SAFETY AND STABILITY OF HIGH PRESSURE PROCESSED BLUE CHEESE DRESSING

A Project Paper

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of Food Science and Technology

by

Lindsey Randall Reardon

May 2018

ABSTRACT

Salad dressings are a staple item for many households. With an increased demand for foods without artificial flavorings, colors, and preservatives, producers are finding ways to innovate and reformulate these products. The purpose of this study was to utilize high pressure processing to reduce the microbiota of blue cheese dressing without compromising product quality. In doing so, the goal was to produce a clean label, shelf-stable dressing without the use of chemical preservatives. After 12 weeks, CFU/g of yeasts and filamentous fungi were below the detectable limit of 10. A 5-log reduction of *E. coli, Listeria, Salmonella* was achieved and maintained after 10 weeks. High pressure processing was an effective technology for producing a good quality, relatively high pH at 4.0, blue cheese dressing with microbiological, physical, and chemical stability.

BIOGRAPHICAL SKETCH

Lindsey Reardon received Bachelor of Science degrees in both Food Science and Nutrition Science from North Carolina State University in 2014. During her studies, she completed process engineering internships at MWV and the Dow Chemical Company, in addition to an R&D internship at Land O'Lakes. Upon graduating, Lindsey worked as a Continuous Improvement Engineer at the Kraft Foods string cheese facility in Campbell, NY. When Kraft Foods merged with the Heinz company, Lindsey was transitioned to a Production Supervisor role at the Campbell, NY facility in which she led the daily operations for over sixty direct reports. After plant closure rumors, Lindsey accepted a position with the J.M. Smucker Company. She moved to Buffalo, NY to work at the Milk-Bone® plant within their Pet Foods Division. Lindsey completed process improvement projects and supplier approvals of incoming ingredients. She also completed Preventive Controls training and helped develop the plant's food safety plan. She then decided to a pursue her graduate degree in Food Science at Cornell University. While at Cornell University, she worked for Dairy Foods Extension and TA'd the undergraduate Capstone course. Her research has focused on exploring the impacts of high pressure processing on a personal formulation for blue cheese dressing. Her goal was to evaluate this as a feasible option for development of a clean label, shelf-stable version of the initial recipe.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Olga Padilla-Zakour, who may not have planned on taking on any M.P.S students this year, yet she didn't even hesitate when approached about being a potential advisor. Not only that, but she allowed me to design my own my project, while giving me direction on how to arrange my study, what data to collect, and how to interpret my results. She has been gracious in setting me up with resources in Geneva, with industry partners, and potential employers. I also thank her for her kindness and compassion throughout the semester and for always taking an interest in what is going on.

Kyle Kriner was extremely instrumental in helping me get my project off the ground. Thank you for showing me how to use multiple pieces of equipment and for helping me solidify my methods for analytical testing. Not only that, but you made yourself available to answer questions at any point in time. Thanks for always checking in to make sure things were progressing well.

I would also like to thank Dr. Randy Worobo for providing me valuable insights about blue cheese, commercialization, and spoilage. Between Dr. Worobo and Liz Buerman, I was able to "refresh" my food microbiology knowledge and plating skills. Thanks to Liz for answering all questions related media, specific organisms, counts, HPP, data analysis, etc.

John Churey and Andy Humiston at the HPP facility in Geneva were flexible with my schedule, helped me operate the HPP unit, and allowed me to work in their space to complete my plating. I would like to give a huge thank you to John, for educating me about handling pathogens and for plating my samples more than once when I wasn't available to come to Geneva.

Thanks to Avery Becker for training me in PCR and sequencing, and for running multiple samples for me to identify a specific organism for my research.

LiDestri Food & Beverage allowed me to come on-site during normal production to process my samples in their HPP unit for sensory testing. Another facility that I utilized multiple times for data collection was the Cornell University NMR Processing Center.

Thanks to Alina Stelick and Anna Scheglov for their assistance in getting my sensory study set up and properly carried out. Aaron Jacobson was also helpful in providing me microbiology insights and for permitting me to utilize his teaching lab space.

Lastly, thanks to the rest of my lab group for helping in one way or another, whether filling samples, sharing methods, or just being a friend (Rohil Bhatnagar, Junyi Chen, Nathan Feist, Julie Flinois, Kyle Kriner, Kate Pinsley, Emile Punzalan, Shuang Qiu, and Sandy Thai).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Blue cheeses have likely been produced for several years, either deliberately or by accident. There are writings that describe different varieties dating as far back as 879 (Cantor, et al., 2004). This style of cheese can be consumed by itself, as a spread, or melted atop something else. On store shelves today, consumers can also find a crumbled form of blue cheese, which breaks down the cheese loaf before packaging. Blue cheese has also become popular as a dip or dressing, in which blue cheese crumbles are suspended in a creamy matrix. These dressings are sold refrigerated or shelf-stable for 3 to 6 months. Producers use preservation hurdles that allow for these products to be stored at ambient temperature for extended periods of time. High-pressure is a processing technique that is being studied for application to sauces, such as blue cheese dressing. The purpose of this study was to utilize high pressure processing to reduce the microbiota of blue cheese dressing without compromising product quality. In doing so, the goal was to produce a clean label, shelf-stable dressing without the use of chemical preservatives.

Blue Cheese

Blue vein style cheeses are produced by inclusion of the fungi *Penicillium roqueforti.* They are well known for their sharp, tangy flavor and characteristic ammonia-like odor. The most common varieties of blue cheeses include: Gorgonzola, Roquefort, Stilton, and Danblu. While they may be produced using different processes (i.e. goat's milk, cow's milk, brine salting, dry salting, etc.), the most important element includes the mold ripening at the surface (Cantor et al., 2004).

The *Penicillium* culture is either added directly to the cheese's milk or sprinkled on the curd before formation of a loaf finished product. A starter culture of *Streptococcus Lactis* is also added to the milk with rennet for coagulation. Initial fermentation is performed by the lactic acid bacteria (LAB). The LAB will eventually be killed by the low pH, and the Penicillium roqueforti will take over to ferment and break down the lactic acid, driving the pH above 6.0. The pH can

rise even further from the loss of lactic acid. The curds are formed into a loosely packed loaf to be subsequently injected with a needle. This process allows air to penetrate the interior of the loaf, feeding the mold cultures. The needling process will dictate the final appearance of the marbled blue veins throughout the cheese loaf. Cool ripening temperatures and a high relative humidity atmosphere are maintained for favorable mold growth (Nelson, 1970).

After the formation of mold growth and proper aging, product will be packaged into containers and sent for retail sale. Enzymes that carry out lipolysis and proteolysis may remain active and continue to ferment. Packaged blue cheese crumbles would be incorporated into an acidic mayonnaise base with spices, and then repackaged as a blue cheese dressing. Pourable dressings are a greater than $1 billion industry (Anonymous, 2006). With no standard of identity, composition of these dressings can vary greatly. Mayonnaise and mayonnaise-based dressings are formulated with thickening and gelling agents that are often polysaccharide and protein hydrocolloids. They are also formulated with emulsifiers and sometimes stabilizers (Sikora et al., 2008). Spoilage of these dressings can result from separation of the emulsion, oxidation and hydrolysis of the oils, or microbiological growth (Frazier, 1967).

Blue Cheese Dressing Quality and Safety

Blue cheese dressings are generally 50-75% mayonnaise by weight. The main cause of spoilage in mayonnaise-based products is yeasts and bacteria. Molds are capable of growing in these products, yet the occurrence is rare and thought to be due to air contamination (Smittle & Flowers, 1982). In a study by Appleman et al., a species of *Saccharomyces* and *Bacillus subtilis* were both abundant in spoiled mayonnaise (Appleman et al., 1949). A follow up study by Kurtzman et al. isolated and identified the sources of spoilage across a number of mayonnaise and salad dressing samples. *Saccharomyces bailli* yeast was determined to be present in two-thirds of the spoiled samples that were examined (Kurtzman et al., 1971). This organism poses a universal problem in the salad dressing industry because it is acid tolerant, osmophilic tolerant, and resistant to preservatives (Smittle & Flowers, 1982). Others were spoiled by *Lactobacillus*

fructivorans. Small numbers of bacilli were seen as well (Kurtzman et al., 1971). While lactobacilli do not present a human health hazard, they can alter sensory properties.

Survival of pathogenic bacteria in mayonnaise and salad dressings has not generally presented a health hazard. Four strains of coliform, indicators of possible contamination, were investigated for survival in mayonnaise and ranch dressing. The results indicated a greater antimicrobial effect in mayonnaise than in the ranch dressing, "which might be attributed to differences in pH, water activity, nutrients, storage temperature and the presence of lysozyme in the whole eggs, used in its production" (Sikora et al., 2008). Although growth of the coliform wasn't supported in either mayonnaise or ranch dressing, greater survival was observed in the refrigerated dressing than the mayonnaise (Raghubeer et al., 1995). Guerzoni et al. measured the survival of *Salmonella enteritidis* in pressure treated mayonnaise products in relation to NaCl content and pH. Modelling of the survival indicated a synergistic effect between these two variables and levels of *Salmonella enteritidi* (Guerzoni, 2002). An inoculation study showed death of *Salmonella, Escherichia coli O157:H7, E. coli, L. monocytogenes, Staphylococcus aureus,* and *Yersinia enterocolitica* when injected into mayonnaise and dressings (Smittle, 2000).

The introduction of spoilage microorganisms has often been reported as coming from contaminated ingredients or unsanitary manufacturing equipment and surroundings. Studies have been performed to understand and optimize pasteurization temperatures for controlling microbial growth in oil-water mixtures. There is more to be understood regarding other control techniques in the processing and packaging of these types of food products (Kurtzman et al., 1971). One interesting thing to note is that these products are characteristic of undergoing a delayed spoilage. The yeasts and lactobacilli that have been isolated from spoiled salad dressings are rapid fermenters of fructose. Since sucrose is slowly hydrolyzed by acids into glucose and fructose, the sudden onset of spoilage may be explained by the onset of fructose fermentation (Smittle & Flowers, 1982).

Preservation Methods for Dressings

In the U.S., mayonnaise is defined as containing ≥65% vegetable oil, acidifying agents, egg yolk or whole eggs, seasonings, color and/or flavor stabilizers, citric and/or malic acid and crystallization inhibitors (Smittle & Flowers, 1982). It falls under the regulations for acid foods, with a pH falling between 3.0-4.2 and 4.5 being the legal maximum. No regulation is set forth for the sodium content, but typical ranges fall between 1% to 12% in the aqueous phase, with a resulting water activity between 0.95 to 0.93 (Vermeulen, 2008). Although salad dressings can greatly vary, they are often derivatives of mayonnaise turned into an emulsified semisolid food.

Acidulants. Two major groups of acids that can be applied to salad dressings for preventing and controlling growth of unwanted organisms: acidulants and preservatives. Acidulants are generally added in larger quantities, and these include hydrochloric acid, acetic acid, lactic acid, and citric acid. The functionality of acidulants relies on the release of protons. It is thought that the undissociated form of these acids is what is responsible for the antimicrobial activity. The acidification effects are dependent on both the strength of the acid and pH of the medium they are being added to (Vermeulen, 2008).

When comparing equal molar concentrations, citric acid results in greater activity than acetic acid. However, acetic acid has proved to have greater antimicrobial activity when compared across equal pH values (Sorrells et al., 1989). It can also be used at lower concentrations than lactic acid or HCl to achieve the same results. Acetic acid is arguably the most widely used acidulant applied to foods, especially in mayonnaise, dressing, and sauces. Major producers utilize it at 0.90-0.928% for salad dressings (Smittle, 1977). The concentration level that can be used is a consideration, because its pungency will have impacts on odor and taste (Vermeulen, 2008). Acetic acid has proved to be effective in the quick destruction of vegetative cells. Although spores will remain viable, acetic acid can still inhibit growth if germination of spores is to occur (Smittle, 1977).

Chemical preservatives. Chemical preservatives are added in smaller quantities, and these include sorbic acid and benzoic acid. Their mechanism of action is destruction of cell

membranes and interruption of the glycolytic energy cycle (Piper et al., 2001). The activity potential of sorbic acid and benzoic acid is dependent on the structure of compounds within a food, pH, and moisture content. They are most effective as an antimicrobial towards yeasts and molds. They are not very effective in inhibiting the growth of lactobacilli alone, but they can act in combination with acidulants (Fialova et al., 2007). Although they do not have a significant impact on flavor or aroma of food products, there are some limitations with using chemical preservatives. Sorbate can degrade at higher temperatures, benzoic acid has a narrow pH range at which it is effective, and there are regulatory maximum allowable concentrations for both chemicals (Vermeulen, 2008). The Dictionary of Food Ingredients states that the maximum usage level for sorbates and benzoates is 0.01% (Igoe, 2011).

Water activity. Another control method against microbiological growth in salad dressings is reduced water activity. When compounds such as salt or sugar are solubilized in food solutions, they decrease the amount of available water for bacteria to use. They also cause osmotic stress on cells, causing rapid water loss, or plasmolysis, internally. During this process, cells will not grow, so they will either die or remain dormant. In order to grow, these cells will either need to take in some solute or produce their own solute to reduce their intracellular water activity (Sperber, 1983). As previously mentioned, mayonnaise has a water activity of ~0.925, which is equivalent to 12% NaCl. Salad dressings average a water activity of ~0.929 (Smittle, 1977). In a study by Meyer et al., yeasts were able to grow in dressings as low as 0.89, but *L. fructivorans* was only able to grow at a water activity >0.95 (Meyer, 1989). When combined with a reduced pH, reduced water activity exhibits inhibitory properties against both spoilage organisms and pathogens.

Pasteurization. Lastly, heat treatment is a common processing technique applied to salad dressings prior to packaging. Foods with a pH of 4.6 or below must be processed to achieve a 5-log reduction in pathogens. Heat is thought to inactivate yeast cells by damaging the membrane, ribosomes, and mitochondria (Rai & Bai, 2014). Acid dressings undergo sterilization to produce a shelf stable product, while low acid sauces undergo pasteurization and produce and product

that must be refrigerated. When sterilization by hot fill and inversion is performed, product packaging type is a consideration. There is a lack of peer-reviewed documentation to support the FDA's guidance for processing conditions of products with a pH of 4.1-4.6. Breidt et al. developed thermal destruction models for *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in acid foods. The thermal recommendations were based off findings that *Listeria monocytogenes* is the most heat resistant. Among the range of recommended time-temperature combinations, processing for 20 minutes at 67°C resulted in a 5-log reduction (Breidt, 2014).

High Pressure Processing

There has been a growing consumer demand for not only fresh foods, but clean label products that still retain certain sensory characteristics within a reasonable shelf-life. Traditional thermal methods used for preservation purposes often have negative impacts on sensory attributes, flavors, and nutritional contents (Huang et al., 2016). Many times, they also still need to be used in conjunction with chemical preservatives to address spoilage organisms that the thermal treatment does not kill. For this reason, many non-thermal technologies, including pulsed electric field, pulsed light, electron beam, plasma, and modified atmosphere packaging are being thoroughly investigated to meet the demands of consumers. One non-thermal processing technique that has been successfully applied to food commercially is high-pressure processing (HPP) (Huang et al., 2016). During the process, food is filled and hermetically sealed into high pressure resistant packaging, loaded into a water filled chamber and subjected to isostatic pressure between 100-600 MPa (Balasubramaniuam et al., 2015). The entire process is carried out at refrigeration or milk process temperatures (<45°C) (Mircea-Valentin Muntean et al, 2016).

High-pressure processing was proven successful in food as earlier as 1899, when Hite investigated its use in killing bacteria in milk for preservation purposes. It wasn't until eighty years later that a Japanese company revisited the technology and launched the first commercial

product, a jam. The research into the benefits of this technology has come so quickly, that many other products have launched since, including fruit juices, guacamole, and oysters (Rastogi et al., 2007). Many ready-to-eat meat products are undergoing HPP to both eliminate *Listeria monocytogenes* and strengthen enzymes that accelerate the aging and tenderization process. Several juice producers are implementing the process to increase the shelf-life of their products by more than 3-fold while retaining flavor profiles. Avocados benefit from the suppression of polyphenol oxidase, which is responsible from enzymatic browning if active (Huang et al., 2016). Oysters placed under pressures between 240-350 MPa have a denatured abductor muscle, allowing them to be opened without needing to be handshucked. They also reap the benefits of the reduction of *Vibrio*, while still retaining raw qualities. The technology is also being studied for things like improving tactility of cheese for shredding, reduction of protein damage in seafood, and removal of added nutrients from certain foods (Torres & Velazquez, 2004).

The major advantage of applying HPP is that many foodborne pathogens are not able to survive the high-pressure stress. It has been proved to result in 5-log reductions in *Salmonella typhimurium, Salmonella enteritidis, Listeria monocytogenes, Staphylococcus aureus, and Vibrio parahaemolyticus* (Torres & Velazquez, 2004). Not only that, but HPP has been shown to reduce microbiological load of spoilage organisms even without the addition of chemical preservatives (Huang et al., 2016). It inactivates these organisms by damaging their membranes and interrupting their cellular functions. Without the ability to uptake nutrients or dispose of waste, the microorganisms are unable to reproduce and survive (Torres & Velazquez, 2004). Regardless of the high-pressure stress, the covalent bonds in food do not break down when using this technology, conserving the chemistry of the finished products. When compared with heat treated foods, HPP has proven to have minimal impact to taste, texture, appearance, and nutritional value. Another advantage of HPP is that due to the equally distributed pressure, the effects of the treatment are not dependent on packaging form or volume of the food (Torres & Velazquez, 2004). What that means is that foods with different volumes can be processed in the same batch. Even further, since foods are processed in their final packaging, there is no contact with any

surfaces of the processing equipment, reducing the risk of post-processing contamination. Lastly, there is potential for reduced energy consumption when using HPP methods. Since the process is carried out at refrigeration temperatures or ambient temperatures, there is no need for a heating and then secondary cooling process. The pressure transfer medium used within HPP chambers can also be recycled in used in subsequent processing (Huang et al., 2016).

One challenge that HPP presents is that many foods still require refrigerated storage post-processing. This is because pressure alone is not effective in inactivating spores that would be permitted to grow without cold storage, particularly *Bacillus* and *Clostridium* (Huang et al., 2016). Some studies have shown success in inactivation of these spores at higher pressures or longer hold times. Processors also have the option to use pulsed pressure, pressure and temperature combinations, or pressure combined with ultrasonic energy, PEF, or irradiation (Zhang & Mittal, 2008). There are also limitations to what foods HPP can be applied to. Since the technology requires water to be available within a food to act as a pressure transfer medium, HPP is not effective in low water activity foods. In a study by Setikaite et al., the inactivation of *E. coli* decreased by 2.3-log when water activity was brought from 0.99 to 0.95, and then decreased even further when brought down to 0.90. Even further, the solute type used to control water activity in a food can have an impact on HPP effectiveness. Sodium chloride was shown to deliver a 6.03-log reduction in *E. coli* versus a 0.38-log reduction when sorbitol was used to control to the same water activity (Setikaite et al., 2008). Lastly, processors need to be selective in choosing packaging for their products, as any material with a compressibility factor of <15% will not withstand the high-pressure stress (Huang et al., 2016). Many flexible plastics are suitable for the process, but glass or aluminum containers cannot undergo HPP.

There are now more than 10 HPP equipment suppliers in the world, with more than 300 sets of HPP equipment operating for mass production. Major manufacturers include: Avure, Hiberbaric, and Multivac. These manufacturers offer both horizontal and vertical type units, and the cost for a set of equipment ranges from $0.5-2.5 million depending on capacity (Huang et al., 2016). In 2015, the global market for food products processed using this technology reached

about $9.8 billion. With the rising pressures from consumers for fresh foods, it is expected that the market value for HPP products will reach $54.77 billion by 2025 (Visiongain, 2015). It will become increasingly important to continue investigating the impacts of HPP on specific food types to understand impacts to microbial content, shelf-life, and organoleptic properties.

REFERENCES

Anonymous. 2006. 59th annual consumer expenditure study. *Progressive Grocer* 85(13):34.

Appleman, M. D., Hess, E. P., and Rittenbeg, S. C. 1949. An investigation of a mayonnaise spoilage. *Food Technol*. 3:201-203.

Balasubramaniam, V.M., Martínez-Monteagudo, S.I., and Gupta, R. 2015. Principles and Application of High Pressure–Based Technologies in the Food Industry. *Annual Review of Food Science and Technology* 6:435-462.

Breidt, F., Kay, K., Osborne, J., Ingham, B., and Arritt, F. 2014. Thermal Processing of Acidified Foods with pH 4.1 to pH 4.6. *Food Protection Trends* 34(3):132-138.

Cantor, M.D., van den Tampel, T., Hansen, T.K., and Ardo, Y. 2004. Blue Cheese. *Cheese: Chemistry, Physics and Microbiology* 3(2), 175-196.

Fialova, J., Chumchalova, J., Mikova, K., and Hrusova, I. 2007. *Food Control* 19(2008):706-713.

Frazier, W.C. 1967. Food microbiology, p. 537. McGraw Hill, Inc., New York.

Guerzoni, M.E., Vannini, L., Lanciotti, R., and Gardini, F. 2002. Optimization of the formulation and of technological process of egg-based products for the prevention of Salmonella enteritidis survival and growth. *Int. J. Food Microbiol*. 73(2-3):367–374.

Hongkang Z. and G.S. Mittal. 2008. Effects of High-Pressure Processing (HPP) on

Bacterial Spores: An Overview. *Food Reviews International* 24:3, 330-35.

Huang, H.S., Wu, S.J., Lu, J.K., Shyu, Y.T., and Wang, C.Y. 2016. Current status and future trends of high-pressure processing in food industry. *Food Control* 72(2017):1-8.

Igoe, R.S. 2011. Dictionary of Food Ingredients: Fifth Edition. Springer Science & Business Media, LLC.

Kurtzman, C.P., Rogers, R., and Hesseltine, C.W. 1971. Microbiological Spoilage of Mayonnaise and Salad Dressings. *Applied Microbiology* 21(5), 870-874.

Meyer, R.S., Grant, M.A., Luedecke, L.O., and Leung, H.K. 1989. Effects of pH and Water Activity on Microbiological Stability of Salad Dressing. *Journal of Food Protection* 52(7):477-479.

Mircea-Valentin, M., Marian, O., Barbieru, V., Cătunescu, G.M., Ranta, O., Drocas, I., and Terhes, S. High Pressure Processing in Food Industry – Characteristics and Applications. *Agriculture and Agricultural Science Procedia* 10:377-383.

Nelson, J.H. 1970. Production of Blue Cheese Flavor via Submerged Fermentation by Penicillium roqueforti. *Journal of Agricultural and Food Chemistry* 18(4), 567-569.

Piper, P., Calderon, C. O., Hatzixanthis, K., and Mollapour, M. 2001. Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. *Microbiology-Sgm* 147, 2635-2642.

Rai, V.R. and J.A. Bai. 2014. Microbial Food Safety and Preservation Techniques. Technology

& Engineering, CRC Press.

Raghubeer, E. V., Ke, J. S., Campbell, M. L., and Meyer, R. S. 1995. Fate of Escherichia coli 0157: H7 and other coliforms in commercial mayonnaise and refrigerated salad dressing. *J. Food Prot*. 58(1):13–18.

Rastogi, N.K., Raghavarao, K.S., Balasubramaniam, V.M., Niranjan, K., Knorr, D. 2007. Opportunities and challenges in high pressure processing of foods. *Crit Rev Food Sci Nutr*. 47(1):69-112.

Setikaite, I., Koutchma, T., Patazca, E., Parisi, B. 2008. Effects of Water Activity in Model Systems on High-Pressure Inactivation of Escherichia coli. *Food and Bioprocess Technology* 2009, 2:213-221.

Sikora, M., Badrie, N., Deisingh, A.K., and Kowalski, S. 2008. Sauces and Dressings: A Review of Properties and Applications, *Critical Reviews in Food Science and Nutrition* 48:1, 50-7.

Smittle, R.B., and R.S. Flowers. 1982. Acid Tolerant Microorganisms Involved in the Spoilage of Salad Dressings. *Journal of Food Protection* 45(10):977-983.

Smittle, R. B. 1977. Microbiology of mayonnaise and salad dressing - Review. *Journal of Food Protection* 40:415-422.

Smittle, R. B. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: A review. *Journal of Food Protection* 63:1144-1153.

Sorrells, K. M., Enigl, D. C., and Hatfield, J. R. 1989. Effect of pH, acidulant, time, and temperature on the growth and survival of Listeria monocytogenes. *Journal of Food Protection* 52:571-573.

Sperber, W.H. 1983. Influence of Water Activity on Foodborne Bacteria — A Review. *Journal of Food Protection* 46(2):142-150.

Torres, J.A. and G. Valazquez. 2004. Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering* 67(2005):95-112.

Vermeulen, A. 2008. Microbial stability and safety of acid sauces and mayonnaise-based salads assessed through probabilistic growth/no growth models Thesis submitted in fulfillment of the requirements for the degree of doctor (Ph.D.) in Applied Biological Sciences. Faculty of Bioscience Engineering, University of Ghent.

CHAPTER 2

Abstract

HPP was applied to a formulation of blue cheese dressing to evaluate the efficacy of its use as an alternative to thermal processing. Three variables were altered in an initial study to optimize the final formulation. These included pH (4.3 and 4.0), water activity (0.965 and 0.950), and low vs. high vein blue cheese crumbles. The blue cheese was processed using a Hiperbaric 55 high-pressure processor (HPP) at the maximum pressure of 87,000 pounds per square inch for 2 minutes. Based on microbiological results, it was decided to move forward with a formulation of pH 4.0, water activity 0.950, and low vein blue cheese crumbles.

The selected formula was high-pressure processed under the same parameters. It was evaluated over shelf-life at 4° and 22 °C for microbiological growth, pH, viscosity, particle size, lactic acid, acetic acid, and citric acid. After 12 weeks, CFU/g of yeasts and filamentous fungi were below the detectable limit of 10. Additionally, it was tested for color, sodium content, and a pathogen challenge study was performed. A 5-log reduction of *E. coli, Listeria, Salmonella* was achieved and maintained after 10 weeks. The HPP treated blue cheese dressing was compared against an ambient filled formulation with preservatives and a pasteurized formulation with preservatives for sensory testing. High pressure processing was an effective technology for producing a good quality, relatively high pH at 4.0, blue cheese dressing with microbiological, physical, and chemical stability for at least 12 weeks at 4 °C and 9 weeks at 22 °C.

Introduction

High-pressure processing has proven to be an effective technology for reduction or elimination of microorganisms in food without deteriorating product quality. While it was first

commercialized in jam products, and it has subsequently been implemented in juice products and packaged avocados, there is more research to be done to study its impact in dressings and sauces. In a study by Waite et. al. (2009), ranch dressing was processed at 600 MPa for 5 minutes and then evaluated over 26 weeks. The study evaluated shelf-life impacts when held at both 4 ℃ and 26 ℃. Researchers concluded that a 6.4 log CFU/g reduction in spoilage organisms was achievable (Waite et al., 2009). This followed up on an earlier study by Nienaber et al. in which HPP effectively inactivated *Z. bailii* and *L. fructivorans* in ranch dressing (Nienaber et al., 2001). In Waite's study, HPP had no effect on emulsion stability or pH. There was a slight increase in viscosity or product thickness as shelf-life progressed. The study revealed decreased consumer acceptance by the end of the storage period (16 to 26 weeks) and adverse color and organic acid profile changes. However, due to the fact that microbiological stability was achieved over time, the researchers concluded that HPP is effective in producing a ranch dressing that is stable for 6 months at refrigeration and 2 months at room temperature (Waite et al., 2009). The objectives of this study were to apply HPP for the production of a safe and stable blue cheese dressing without the use of preservatives and to evaluate its shelf life when stored at refrigeration or room temperature.

Materials & Methods

Ingredients. Food items for the preparation of the blue cheese dressing were purchased from Wegman's Food Market in Ithaca, NY. They are listed in Table 1.

Table 1. Brand and Manufacturer of Food Ingredients Used in Formulation of Blue Cheese Dressing

Ingredient	Brand	Manufacturer
Mayonnaise	Hellman's Real	Unilever, Englewood Cliffs, NJ, USA

Blue Cheese Crumbles	Organic Creamery Organic	Saputo, Inc., Montreal, Canada
Vinegar	Heinz Distilled	Kraft Heinz, Chicago, IL, USA
Half and Half	Upstate Farms	Upstate Niagara Cooperative, Buffalo, NY, USA
Worcestershire Sauce	Lea & Perrins	Kraft Heinz, Chicago, IL, USA
Iodized Salt	Morton	Morton Intl., Inc., Chicago, IL, USA
Garlic Powder	McCormick	McCormick and Co., Inc., Hunt Valley, MD, USA
Black Pepper	Wegman's Pure Ground	Wegman's Food Markets, Rochester, NY, USA

Chemicals and reagents. Preservatives used in the formulation and chemicals and reagents used in analytical testing were purchased from VWR International. They are listed in Table 2.

Table 2. Chemicals and Reagents Used in Blue Cheese Formulation or for Analytical Testing

Ingredient	Purity (%)	Manufacturer
Pre-Hydrated Ticaloid 210 "S"	-	TIC Gums, Belcamp, M.D., U.S.A.
Sodium Benzoate	99.0	Thrasher Hydroponics, Fayetteville, N.C., U.S.A.
Potassium Sorbate	100.0	Chem Products, Portland, O.R., U.S.A.
Peptone	-	BD Difco, Franklin Lakes, N.J., U.S.A.
Tartaric Acid	≥99.0	Fisher Chemical, Hampton, N.H., U.S.A.
TMSP	98.0	Cambridge Isotope Laboratories, Inc., Andover, M.A., U.S.A.
Deuterium Oxide	99.9	Cambridge Isotope Laboratories, Inc., Andover, M.A., U.S.A.
Go Taq Green	-	Promega, Madison, W.I., U.S.A.

Preparation of blue cheese dressing. High pressure processed blue cheese dressing was prepared according to formulation in Tables 3, 4, 5, and 6. All ingredients were added to a stainless-steel mixing bowl and mixed by hand to hydrate the gum and achieve uniformity.

Table 3. Formulation of Blue Cheese Dressing Used for HPP Variable (600 MPa for 2 minutes)

Ingredient	W/w %
Mayonnaise	66.75

Blue Cheese Crumbles	14.75
Vinegar	10.06
Half and Half	5.08
Worcestershire Sauce	1.51
Gum Acacia, Xanthan Gum	0.68
Iodized Salt	0.50
Garlic Powder	0.42
Black Pepper	0.25

Table 4. Formulation of Blue Cheese Dressing Used for Thermally Processed Variable (74 ℃ for 20 minutes)

Ingredient	W/w %
Mayonnaise	66.62
Blue Cheese Crumbles	14.72
Vinegar	10.04
Half and Half	5.07
Worcestershire Sauce	1.50
Iodized Salt	0.50
Garlic Powder	0.42
Black Pepper	0.25
Gum Acacia, Xanthan Gum	0.68
Sodium Benzoate	0.10
Potassium Sorbate	0.10

Processing of blue cheese dressing. Prepared blue cheese dressing was transferred to sterile whirl-pak bags (Nasco, Salida, C.A.) to support all microbiological and sensory and physical testing. The whirl-pak bags were filled with approximately 125 g of dressing to achieve minimal headspace and then induction sealed. Bags were immediately refrigerated until being transferred to the pressure processing facility. HPP was conducted in a 55L volume vessel (Hiberbaric USA, Miami, F.L.). Samples were pressure-processed at 4 ℃ under 600 MPa for 2 minutes.

Table 5. Formulation of Blue Cheese Dressing Used for Control Variable with Preservatives (Ambient Filled with 0.1% Potassium Sorbate and Sodium Benzoate)

Ingredient	W/w %

Mayonnaise	66.62
Blue Cheese Crumbles	14.72
Vinegar	10.04
Half and Half	5.07
Worcestershire Sauce	1.50
Gum Acacia, Xanthan Gum	0.68
Iodized Salt	0.50
Garlic Powder	0.42
Black Pepper	0.25
Sodium Benzoate	0.10
Potassium Sorbate	0.10

Table 6. Formulation of Blue Cheese Dressing Used for Control Variable without Preservatives

Ingredient	**W/w %**
Mayonnaise	66.75
Blue Cheese Crumbles	14.75
Vinegar	10.06
Half and Half	5.08
Worcestershire Sauce	1.51
Gum Acacia, Xanthan Gum	0.68
Iodized Salt	0.50
Garlic Powder	0.42
Black Pepper	0.25

Formulation Optimization. A preliminary study was conducted for the purpose of optimizing formulation variables. Eight total variables were made up and then high-pressure processed in triplicate. The variable factors included: high and low vein blue cheese crumbles (Figure 1), pH of 4.0 and 4.3, and water activity of 0.95 and 0.965. Note that these formulations varied slightly from that listed in Table 3 depending on pH, water activity, and blue cheese crumble type. Samples were plated on lactic petrifilm for LAB, potato dextrose agar acidified at 1% with 10% solution of filtered-sterile tartaric acid for yeasts and molds, MRS agar for LAB, and plate count agar for total microbial counts (Table 7). Based on CFU/g results, it was decided

to move forward with a formulation using low vein blue cheese crumbles, pH 4.0, and water activity of 0.95.

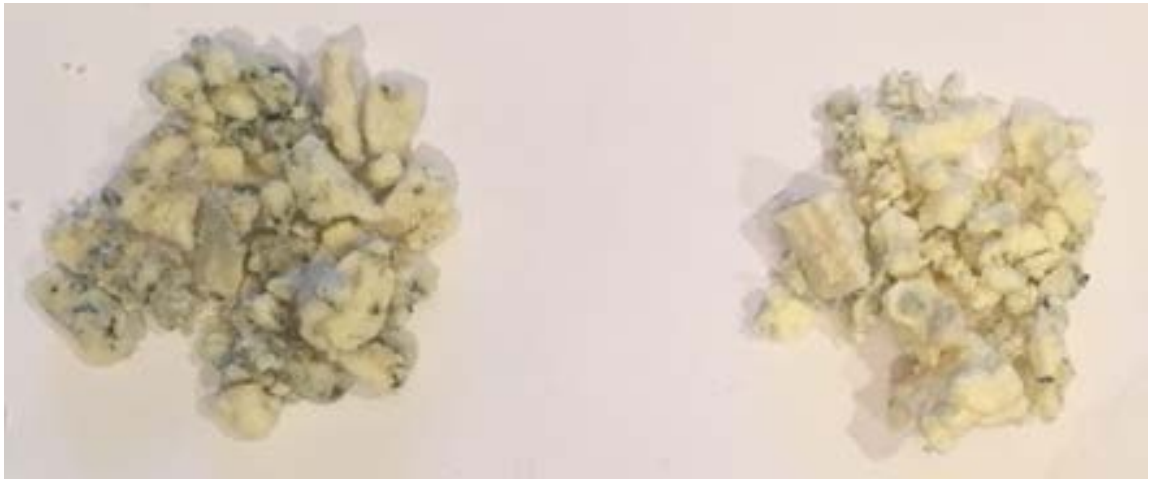


Figure 1. Visual Comparison of High Vein Blue Cheese Crumbles (Left) vs. Low Vein Blue Cheese Crumbles (Right)

Table 7. Various Media Used for Microbial Analyses of Blue Cheese Dressing

Media	Manufacturer
Lactic Petrifilm	3M, Maple Wood, M.N., U.S.A.
Potato Dextrose Agar	BD Difco, Franklin Lakes, N.J., U.S.A.
MRS Agar	BD Difco, Franklin Lakes, N.J., U.S.A.
Plate Count Agar	BD Difco, Franklin Lakes, N.J., U.S.A.
Violet Red Bile Agar	Alpha Biosciences, Baltimore, M.D., U.S.A.)
Bismuth Sulfite Agar	Hardy Diagnostics, Santa Maria, C.A., U.S.A.
Oxford Agar	Alpha Biosciences, Baltimore, M.D., U.S.A

Shelf-life study. After high pressure processing, samples were either transferred to room temperature conditions at 22 ℃ or refrigeration at 4 ℃ based on shelf-life variable. Analyses were scheduled and completed according to the sampling plan summarized in Table 8.

Microbial analyses. For microbial analysis, samples of the dressing were diluted with 0.1% peptone water and plated on lactic petrifilm for LAB, potato dextrose agar acidified at 1% with 10% solution of filtered-sterile tartaric acid for yeasts and molds, MRS agar for LAB, and

plate count agar for total microbial counts (Table 7). Colonies were enumerated following incubation at 30 ℃ for 48 to 72 h.

Table 8. Sampling Schedule for Blue Cheese Dressing Shelf-Life Study

Condition	Product Analysis	Time of Analysis (Week)
HPP (stored at refrigeration)	Microbial (total plate count)	0, 3, 6, 9, 12
	Microbial (lactics)	0, 3, 6, 9, 12
	Microbial (yeasts & filamentous fungi)	0, 3, 6, 9, 12
	Chemical (pH, water activity, organic acids)	0, 3, 6, 9, 12
	Physical (color, viscosity, emulsion stability)	0, 3, 6, 9, 12
HPP (stored at room temperature)	Microbial (total plate count)	0, 3, 6, 9
	Microbial (lactics)	0, 3, 6, 9
	Microbial (yeasts & filamentous fungi)	0, 3, 6, 9
	Chemical (pH, water activity, organic acids)	0, 3, 6, 9
	Physical (color, viscosity, emulsion stability)	0, 3, 6, 9
Ambient fill (stored at refrigeration)	Microbial (total plate count)	0, 1, 2
	Microbial (lactics)	0, 1, 2
	Microbial (yeasts & filamentous fungi)	0, 1, 2
	Chemical (pH, water activity, organic acids)	0, 1, 2
	Physical (color, viscosity, emulsion stability)	0, 1, 2
Ambient fill (stored at room temperature)	Microbial (total plate count)	0, 1, 2
	Microbial (lactics)	0, 1, 2
	Microbial (yeasts & filamentous fungi)	0, 1, 2
	Chemical (pH, water activity, organic acids)	0, 1, 2
	Physical (color, viscosity, emulsion stability)	0, 1, 2

pH. The pH of the dressing was measured with a Thermo Electron Corporation Orion 3 Star pH meter (Thermo Fisher Scientific, Waltham, M.A., U.S.A.).

Water activity. Water activity was measured with a 4TE Dew Point Water Activity meter (Aqua Lab Technologies, Riverside, C.A., U.S.A.).

Viscosity. Viscosity measurements were determined using a Brookfield DV-III Ultra Programmable Rheometer (Brookfield Engineering, Middleboro, M.A., U.S.A) fitted with the

V74 spindle. Temperature was monitored during measurement and the program utilized was 200 rpm for 30 seconds.

Emulsion stability and droplet size. Refractive index measurements were conducted by placing a small sample of dressing onto a Leica Auto Abbe benchtop refractometer (Leica Camera, Wetzlar, Germany). These values were inputted into a Malvern Mastersizer Hydro 2000G laser diffraction particle size analyzer (Malvern Instruments, Malvern, U.K.) and included in calculations to determine particle size distribution.

NMR analysis. Organic acids (acetic acid, lactic acid, and citric acid) were measured using a Bruker AV-500 nuclear magnetic resonance spectroscope (Bruker Corporation, Billerica, M.A., U.S.A.) equipped with a broadband cryoprobe. Samples were prepared by weighing 0.25 g blue cheese dressing into a Fisherbrand mini centrifuge vial (Fisher Scientific, Pittsburgh, P.A., U.S.A.) pipetting 1 ml of deionized water into the vial, and centrifuging in a VWR Model V (VWR Scientific, Radnor, P.A., U.S.A.) at 3,000 rpm for 2 minutes. The mass of the deionized water was recorded. Two to three drops of the resulting liquid layer were capillary pipetted into a glass vial (Kimble Chase, Vineland, N.J., U.S.A.) and the weight was recorded. To the glass vial, 700 ml of a standard containing 0.0240% of deuterated trimethylsilylpropanoic acid (TMSP) in deuterium oxide was added. The mass of the standard added to the sample was recorded. The resulting sample with standard was drawn into a 1 ml NORM-JECT syringe (Henke-Sass, Wolf, Tuttlingen, Germany) and then filtered through a 0.22µm polyether sulfone syringe filter (CELLTREAT Scientific Products, Pepperell, M.A.). This was transferred into an 5mm NMR tube (Norell, Inc., Morganton, N.C., U.S.A.). The NMR data acquisition parameters were set to observe hydrogen nuclei with 128 scans, a 30 second relaxation delay, and a 90 degree excitation pulse. Spectral peaks were integrated and analyzed using NMR software (MestReNova, MestReLab, Escondido, C.A.) for quantification of analytes.

Color. Color components of L, a, and b were also measured with a Chroma Meter CR-400/410 (Konica Minolta Sensing America, Ramsey, N.J., U.S.A.). Hue and chroma were calculated from these values.

Salt content. Samples of blue cheese dressing with and without the sodium benzoate and potassium sorbate were sent to Dairy One Inc. Forage Testing Laboratory (Ithaca, N.Y., U.S.A.) for determination of salt content. Mass ratios for Na+ and Cl- were calculated and then averaged together.

Pathogen validation. A cocktail mixture of five strains of *Salmonella* spp. (Hartford, FSL-R9-5494, FSL-R9-5505, FSL-R9-5273, and FSL-R9-5498), *Listeria monocytogenes* (FSL-J1-103, FSL-J1-109, FSL-R9-0506, FSL-R9-5411, and FSL-R9-5506), and *Escherichia coli* O157:H7 (C-7927, ATTC-43890, ATTC-43894, ATTC-43889, and ATTC-35150) was used to perform a challenge study. 0.5 ml of the cocktail mix of each pathogen was inoculated into 50 g of blue cheese dressing. Diluted samples were plated on violet red bile agar for *E. coli*, bismuth sulfite agar for *Salmonella*, and oxford agar for *Listeria* (Table 7). CFU/g were counted pre and post high-pressure processing and then every 2 weeks for samples stored at 4 ℃.

rpoB PCR and sequencing. Isolates were characterized by sequencing a fragment of the 16S rRNA gene as previously described (Huck, et al., 2008). For lysis and DNA preparation, 100 µL of sterile ddH_2O was pipetted into a 0.2 µL PCR tube and placed in a PCR rack. A sterile loop was used to gather a single colony of culture from an MRS plate. The DNA was transferred to the 100 µL of sterile ddH_2O. The PCR tube was microwaved for 90 seconds to lyse the DNA. The PCR tube was transferred to a thermocycyler (2720 Thermal Cycler, Applied Biosystems, Foster City, C.A., U.S.A.) and run at 95 ℃ for 15 minutes, then cooled to 4 ℃. For amplification, a master mix with the primers listed in Table 9 was prepared according to Table 10 and then 24 µL of the mix was transferred to a well plate. 1 µL of lysate was added to each reaction and the well plate was put into the thermocycler at 94 ℃ for 3 minutes, 94 ℃ for 30 seconds, 20 cycles with an AT that decreased by 0.5 ℃ (55-45℃), 20 cycles with an AT at 45℃, 72 ℃ for 1 minute, 72 ℃ for 7 minutes, and then cooled to 4 ℃. A pre-prepared agarose gel tray was loaded into the gel electrophoresis chamber. 3 µL of PCR product was added to each lane, and the gel was run for 25-30 minutes. It was loaded into a GelDoc and run on ImageLab (Bio-Rad Laboratories, Hercules, C.A., U.S.A.) software for DNA analysis. The PCR

product was also submitted for sequencing analysis using KB Basecaller software (Applied Biosystems, Foster City, C.A., U.S.A.).

Table 9. Primers for rpoB PCR Amplification

Primer Name	Primer Sequence (5' to 3')	Application (Genera)
RZrpoBFV1	AARYTIGGMCCTGAAGAAAT	*Bacillus, Paenibacillus*
RZrpoBRV2	TGIARTTTRTCATCAACCATGTG	
RZrpoBFV2	AARYTNGGHCCTGAAGAAAT	*Bacillus, Paenibacillus*
RZrpoBRV2	TGNARYTTRTCATCAACCATGTC	
RZrpoBFV3	AARYTNGGHCCDGARGAAAT	*Bacillus, Geobacillus, Anoxybacillus, Ureibacillus,*
RZrpoBRV3	TGNARYTTRTCRTCRACCATGTG	*Viridibacillus, Paenibacillus, Lysinibacillus*

Table 10. Master Mix Reagents for PCR Amplification

Reagent	Volume per each 25 µL reaction
Sterile water	7.0 µL
Primer Forward	1.25 µL
Primer Reverse	1.25 µL
2X GoTaq Green Master Mix	12.5 µL

Sensory analyses. From the Cornell Sensory Center database, 100 participants were recruited. The study was conducted following all requirements of the Institutional Review Board of Cornell University. The research was conducted in one half day at the Sensory Evaluation Center at Cornell University.

Samples of freshly prepared blue cheese dressing were 1) filled at ambient temperature with the inclusion of potassium sorbate and sodium benzoate 2) high pressure processed in a Hiperbaric 525L under 600 MPa for 2 minutes (LiDestri Foods, Greece, N.Y.) or 3) heated in a water bath for 74 ℃ for 20 minutes. Final samples were presented at room temperature for sensory evaluation. Samples were randomly given to panelists in individual booths equipped

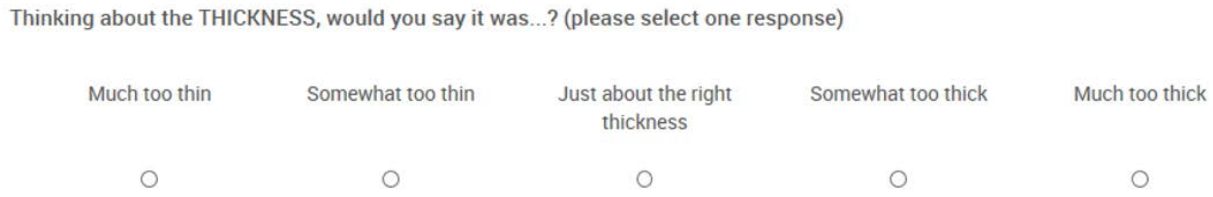
with a computer. The panelists used a 9-point hedonic liking scale (Figure 2) to rate attributes of aroma, color, visual texture, flavor, overall liking, texture, and purchase interest. They used a JAR scale (Figure 3) to rate attributes of saltiness, sourness, and blue cheese intensity. Panelists were also able to include comments on overall likes and dislikes. Following the hedonic scale ratings, panelists were then asked to rank the three samples from most liked to least liked. The

study concluded with asking demographic questions. Sensory management system (RedJade, Curion Corporation, Redwood City, C.A.).

Figure 2. Example 9-point Hedonic Liking Scale Used in Sensory Evaluation

Figure 3. Example JAR Scale Used in Sensory Evaluation

Statistical Analysis. Chemical parameters were subjected to analysis of variance (ANOVA) and significant differences from fresh dressing (time 0) and subsequent shelf-life weeks were analyzed using Dunnett at the 0.05 level. Sensory results were also subjected to



ANOVA and significant differences among treatments were analyzed using Tukey-Kramer HSD at the 0.05 level. Analyses were conducted in Minitab 18 (Minitab Inc., State College, P.A.).

Results and Discussion

Microbial analyses. Samples were plated on acidified potato dextrose agar (APDA), MRS agar, TPC, and lactic petrifilm. The population of yeast and filamentous fungi on APDA in fresh dressing prior to HPP was 5.3 log CFU/g (Figure 4). Treatment with 600 MPa for 2 minutes decreased APDA counts to <1.0 log CFU/g. These counts remained below the detection threshold of 10 CFU/g for the remainder of shelf-life (9 weeks for product held at room temperature and 12 weeks for product held at refrigeration). Research has demonstrated that HPP destroys vegetative cells and studies have shown complete inactivation of spoilage organisms (Han, 2010). Since yeast and filamentous fungi are the concern for spoilage in these types of products, the shelf-life study could have been carried beyond 12 weeks to see if there was any recovery. The control samples started at the same level of log CFU/g, but the sample with preservatives included in the formulation dropped to 4.4 log CFU/g by week 2.

MRS agar favors lactobacilli, but it may allow for growth of other microorganisms (Difco & BBL Manual, 2008). The population of lactobacilli on MRS in fresh dressing prior to HPP was 4.5 log CFU/g (Figure 5). Treatment with 600 MPa for 2 minutes did not totally deplete this population, with post-processing counts being <2.5 log CFU/g. The population on MRS agar remained stable throughout the shelf-life period and did not appear to be growing in HPP treated product. Total plate counts were indicative of the MRS growth, with starting log CFU/g being 3.6 and then remaining fairly stable throughout the shelf-life. This is in line with an HPP study performed on ranch dressing in which lactobacilli counts were reduced by not eliminated (Waite, p. M86). There was no growth at the -1 dilution on 3M lactic petrifilm, so this media may have been too selective for the lactobacilli present in the product.

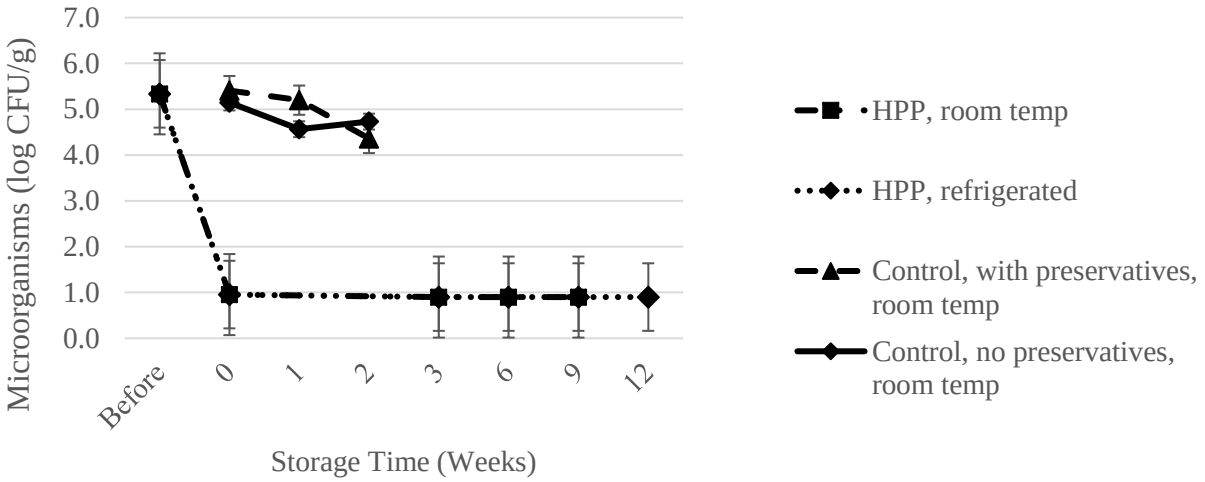


Figure 4. Changes in Yeasts and Filamentous Fungi Growth in Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate.

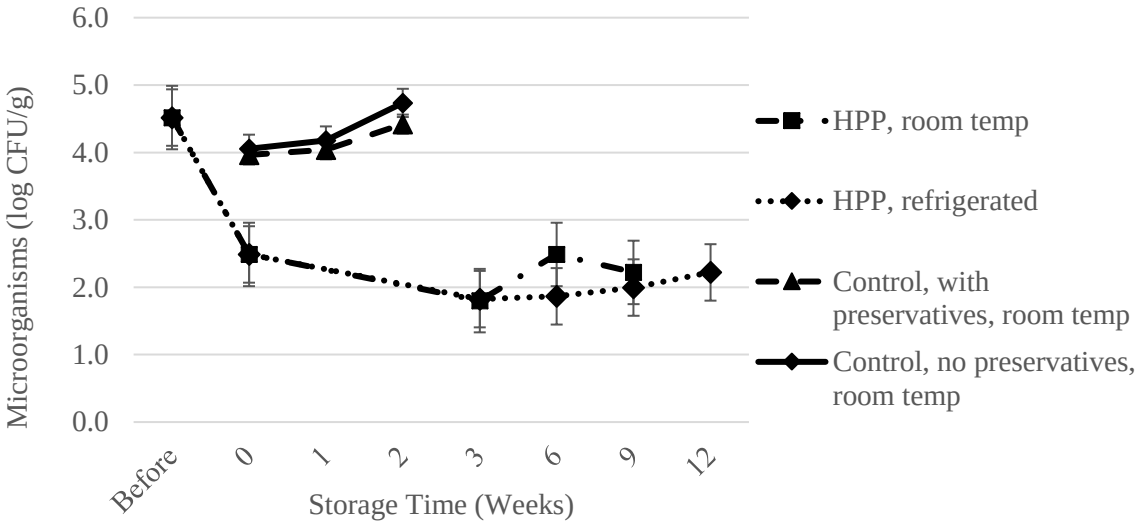


Figure 5. Changes in LAB Growth in Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate.

pH. There was a trend upward in pH in high-pressure processed samples from the starting point of 4.0 before leveling off (Figure 6). This is in line with previous research that shows that

HPP can cause an initial increase in pH due to pressurization of buffers (Patterson et al., 1995; Smelt, p. 1998). Refrigeration conditions kept the product pH lower than those stored at room temperature, so it could be slowing some sort of reaction. It is unknown whether the *Penicillium roqueforti* in the blue cheese crumbles is still active, but the slight increase in pH could be attributed to an equilibrium of the system, because blue cheese itself has a pH <6.0 and it is suspended in an acidic environment.

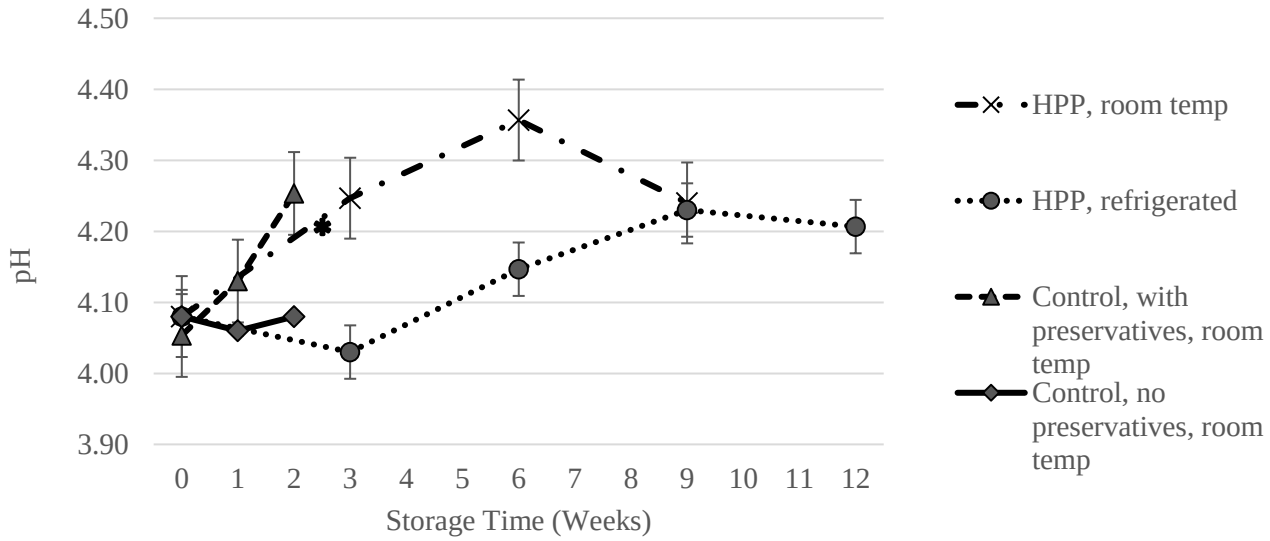


Figure 6. Changes in pH of Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate.
An asterisk (∗) indicates a significant difference ($P < 0.05$) between the stored sample and the initial (time 0) sample.

Water activity. Water activity started at 0.95 and remained stable across shelf-life (Figure 7). There were no statistically significant differences between any variable at time 0 and the stored samples. The slight drop in water activity could have been attributed to equilibrium in the system or moisture migration from the dressing to the blue cheese crumbles.

Viscosity. Viscosity started at 7000 Pa·s in all samples and then remained stable throughout shelf-life, with the exception of it trending down between week 9-12 (Figure 8).

There were no statistically significant differences between any variable at time 0 and the stored samples. A previous study reported that HPP treatments impart a significant increase in viscosity of ranch, French, and slaw dressings after processing (Nienaber et. al, 2001). This trend was not observed in the current study, so rheological changes are likely dependent on formulation. Regarding shelf-life changes, loss of viscosity during storage is typical of most emulsions, regardless of processing treatments (Zablocki et al., 2000). Researchers have reported a decreased in ranch dressing viscosity with extended storage at 26 weeks, although the current study did not extend shelf-life that far (Waite et al., 2009).

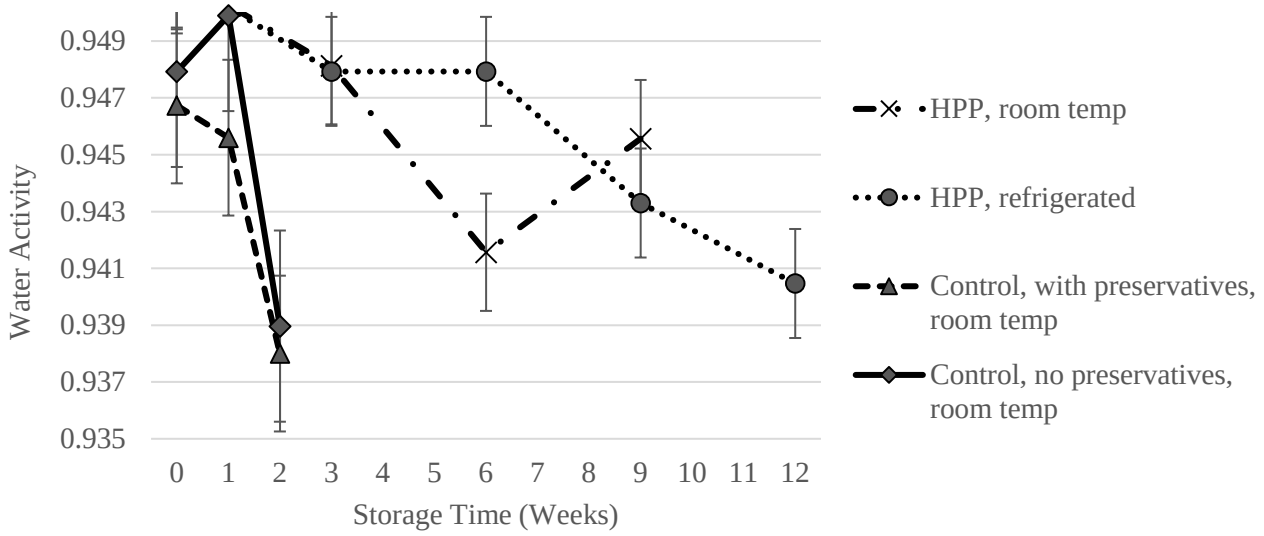


Figure 7. Changes in Water Activity Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. No significant difference ($P < 0.05$) between the stored sample and the initial (time 0) sample was observed.

Emulsion stability and droplet size. The control samples demonstrated a significant increase in volume-averaged geometric mean diameter by week 2 of shelf-life (Figure 9). However, the high-pressure processed samples demonstrated no statistical change in particle size throughout shelf-life. This is representative of emulsion stability. Since oil separation will result in a loss of viscosity due to the breakdown of starches and gums, the current study's droplet size

results are in line with the viscosity results (Claesson et al., 2003; Ford et al., 2003). Emulsion instability can result from either flocculation (rearrangement of oil droplets resulting in separation of fat or proteins from bulk phase) or coalescence of fat droplets (Fetzek, 1973; Mistry and Min, 1993). Other studies by Nienaber et al. and Waite et al. also showed no significant changes in particle size distribution among HPP treated dressings.

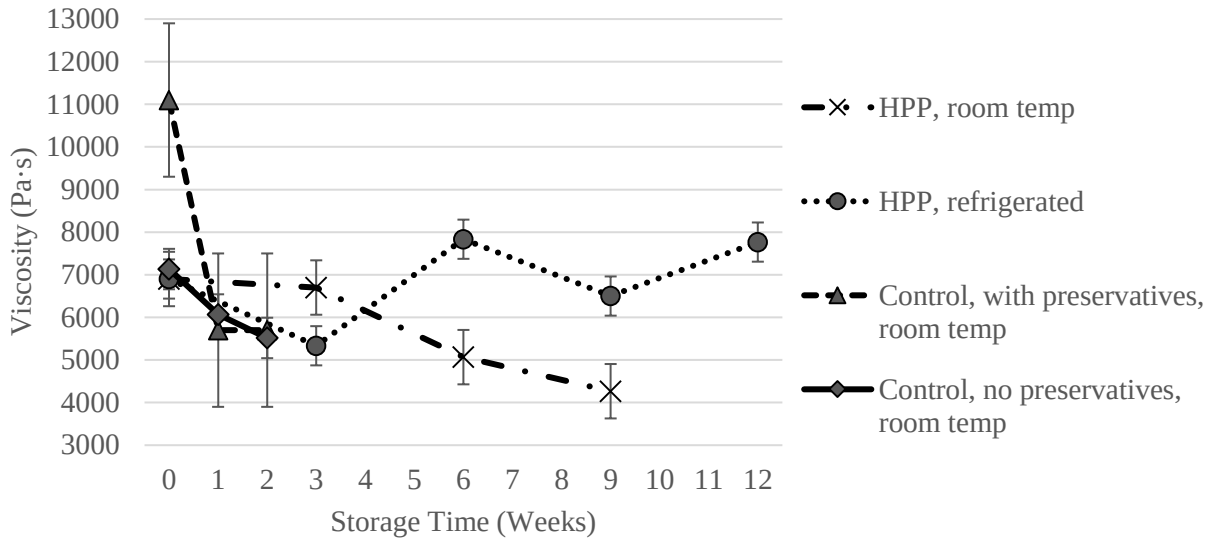


Figure 8. Changes in Viscosity of Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. No significant difference ($P < 0.05$) between the stored sample and the initial (time 0) sample was observed.

NMR analysis. Spectra was used to calculate analyte % based off purity, mass, molecular weight, # of protons, and integration area. Citric acid % remained stable in the product throughout shelf-life. The ingredients of the mayonnaise used to make the product contain citric acid. Both acetic acid % and lactic acid % increased at both the week 1 and week 2 mark in control product (Figure 10). It appeared to be unchanged in HPP produced dressing. These results are in line with the microbial analyses, and it is likely that spoiled product contains

acetobacter, which produces acetic acid and/or lactic acid producing bacteria. Few studies have quantified analyte concentrations of acids post HPP-processing; however, Waite et al. saw modest changes in organic acids throughout shelf-life, other than an increase in those stored at a higher temperature of 37 ℃ (Waite et al., 2009).

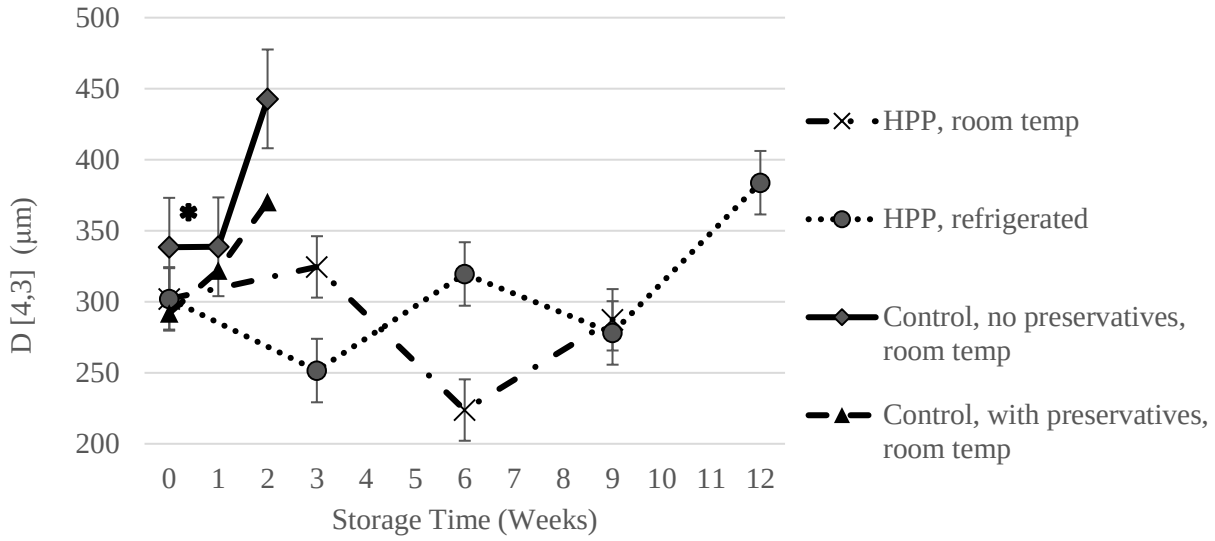


Figure 9. Changes in Particle Size of Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. An asterisk (∗) indicates a significant difference ($P < 0.05$) between the stored sample and the initial (time 0) sample.

Color. L values are indicative of color from black (L=0) to white (L=100). In control samples, L values were significantly lower by week 1 and week 2 (Figure 11). This change was perceivable by the naked eye as a slight yellowing or darkening in color. Color change in salad dressings is not uncommon and may result from the oxidation of carotenoids present in the egg yolk (Weiss, 1983; Fetzek, 1973). In HPP samples stored at both refrigeration and room temperature, there were no significant changes in L value until week 12, where it started to trend down to match the control samples. Color changes are a concern that should be looked at beyond

the 12-week mark, as they can be an indicator of end of consumer acceptance and shelf-life from a quality standpoint.

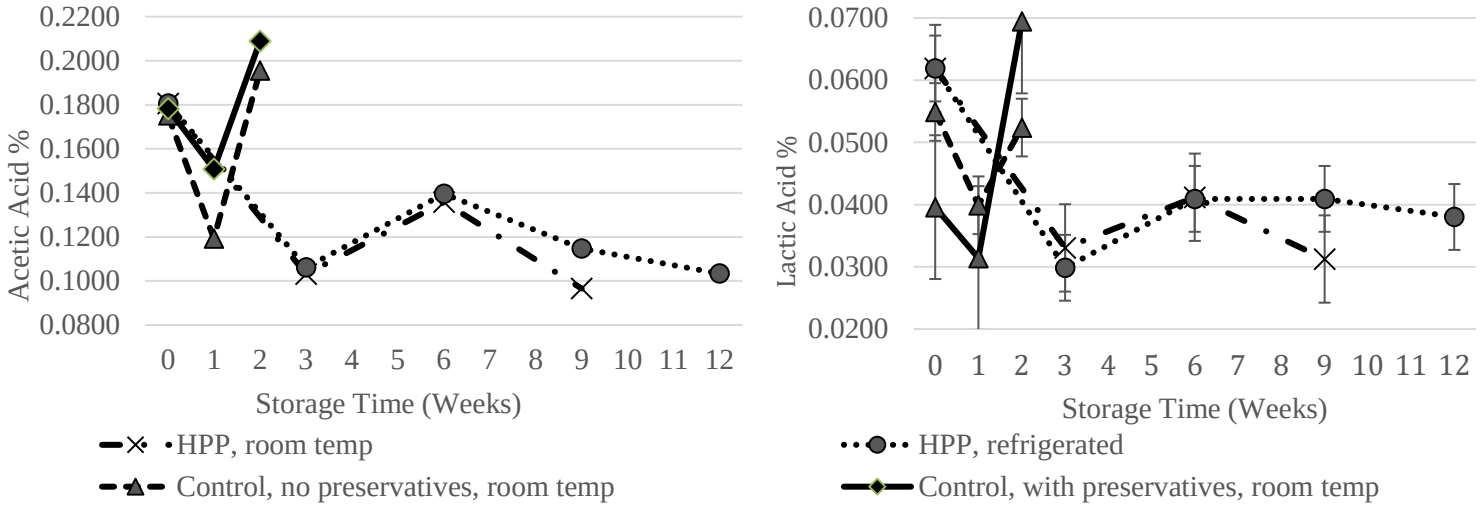


Figure 10. Changes in Acetic Acid & Lactic Acid Concentration in Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. No significant difference ($P < 0.05$) between the stored sample and the initial (time 0) sample was observed.

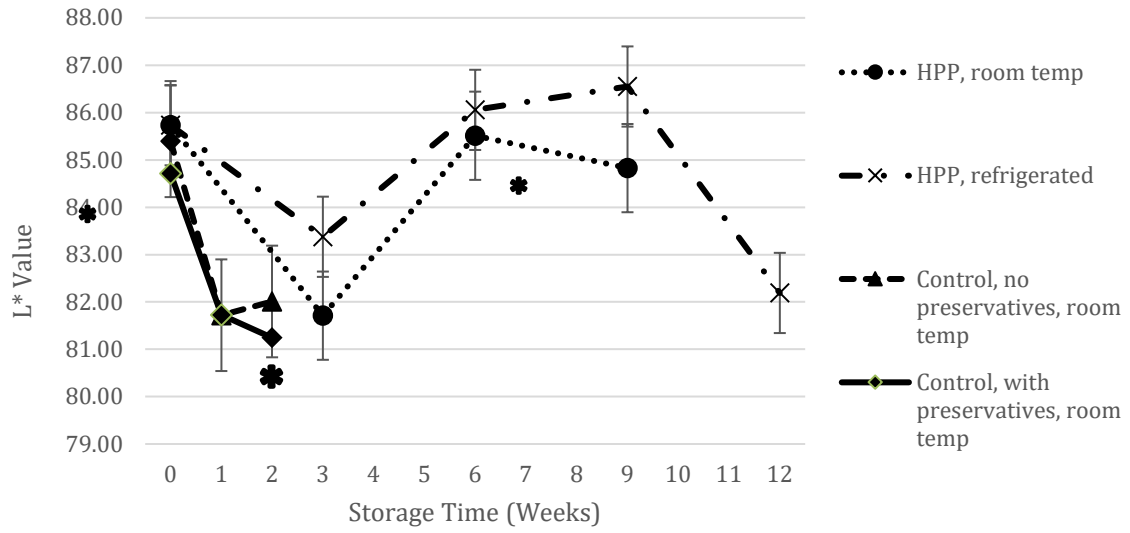


Figure 11. Changes in Color (L-Value) of Blue Cheese Dressing Stored up to 12 weeks at either 4℃ or 22 ℃. The HPP variables were treated at 600 MPa for 2 minutes. The control samples

were filled at 22 ℃ and their formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. An asterisk (∗) indicates a significant difference ($P < 0.05$) between the stored sample and the initial (time 0) sample.

Salt content. Average NaCl content of the formulation was 2.03%. This is of importance in considering growth limits of potential organisms in the dressing. The average sodium content for mayonnaise is reported as 5.43% (Henney et al., 2010). While sodium content can act as a hurdle against the growth of spoilage microorganisms, *Bacillus* species have been reported as able to grow up in 12% NaCl concentrations (Ruiz-Garcia, 2005). As mentioned previously, solute effects also have implications on the effectiveness of HPP. No studies were found that consider the effects based on actual concentration level, but it is known that sodium as a solute in foods accepts HPP effects better than sucrose.

Inoculated studies. Although pathogens are generally not a concern in salad dressings due to their acidic nature, high-pressure processing is unable to inactivate spores (Smelt, 1998). Even though pH conditions do not warrant the activation of spores, the CFR114 states that processors must apply pasteurization or an equivalent process if the pH is close to the cut-off (4.6). The FDA expectation is that there is a 99.999% reduction in pathogens, or a 5-log reduction. In the current study, a 7.5-log reduction was achieved for *Salmonella, Listeria,* and *E. coli.* At 2-week, 6-week, and 10-week shelf-life pulls, at least a 5-log reduction was still maintained. A recent review reported that there are large differences in sensitivity to HPP among pathogenic bacteria. Studies have reported reduction of these organisms in the range of 0.5-8.5 log units (Rendueles, 2011).

rpoB PCR and sequencing. A colony from the growth on the MRS agar was identified by analyzing the genetic coding. The strain was identified by PCR and electrophoresis as *Bacillus velezensis.* It is a spore former that is phylogenetically similar to *Bacillus subtilis*, which has been commonly reported as a spoilage organism in mayonnaise and salad dressings (Appleman et al., 1949). It is able to grow between 15 ℃ and 45 ℃, but its growth should be inhibited by refrigeration temperatures. *Bacillus velezensis* should not grow below a pH of 5.0, which is

likely why it remained stable throughout shelf-life. This strain of *Bacillus* is being study as a novel organism for producing lipopeptides that have antimicrobial properties (Ruiz-Garcia, 2005). However, it shouldn't be synthesizing these metabolites if inactive in the blue cheese dressing.

Sensory analyses. There was no statistical difference in overall liking between the control (ambient filled) blue cheese dressing (6.71) and the high-pressure processed dressing (6.66) (Figure 12). This is in line with research that suggests that HPP has minimal effect on low molecular weight molecules, so flavor compounds and pigments generally go unchanged (Muntean, 2016). There was a statistical difference, however, for overall liking of the heat-treated variable, which scored much lower (4.88). It is known that heat denatures proteins and alters textural properties as a result. Regarding appearance of color, appearance of texture, and aroma, there were no statistical differences between control and the HPP variable. Once again, the heat-treated variable was rated lower on these 9-pt. scales and was statistically different. After tasting the product, panelists rated all three treatments >6.00, with no statistical differences, but the texture of the heat-treated product was rated significantly lower than the control and HPP variables. On the JAR scales, the majority of participants rated thickness as "just about right" for both the control and HPP, and sourness and saltiness as "just about right" for all three variables (Figure 13). While heating the blue cheese did not seem to impart any differences in the flavor profile, it did result in an unfavorable texture and visual appearance, with some actual separation apparent. In the open comments, many participants commented about their fondness for the smoothness, thick texture, and large chunks of blue cheese. Some were sensitive to the sourness or acidity, likely due to the level of added vinegar. Waite et al. reported a decrease in consumer liking of ranch dressing processed under HPP conditions, but not until weeks 16 to 26, but the current study did not study consumer acceptance at the end of shelf-life.

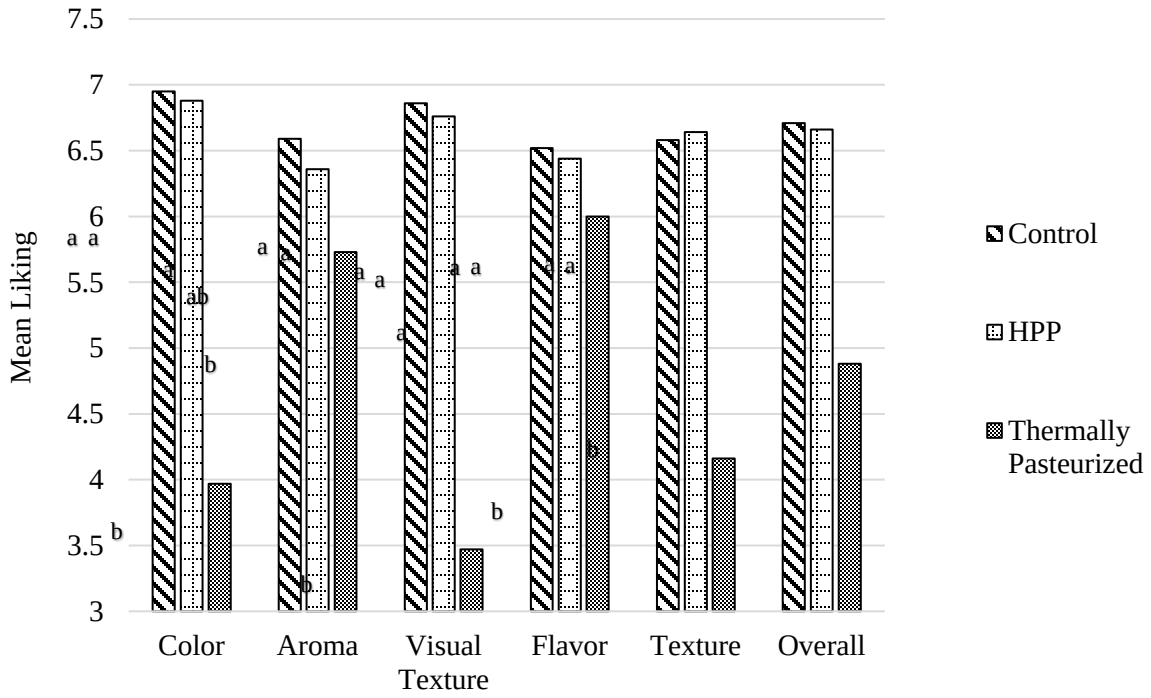


Figure 12. Mean Liking of Various Sensory Attributes of Treated Blue Cheese Dressing (HPP and Thermally Pasteurized) Against Control. A 9-pt. scale from "dislike extremely" to "like extremely" was used. The HPP variables were treated at 600 MPa for 2 minutes. The control variable was filled at 22 ℃ and its formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. The thermally pasteurized variable was treated at 74 ℃ for 20 minutes and its formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. Difference values between treatments not connected by the same letter are significant ($p < 0.05$) by Tukey Kramer HSD.

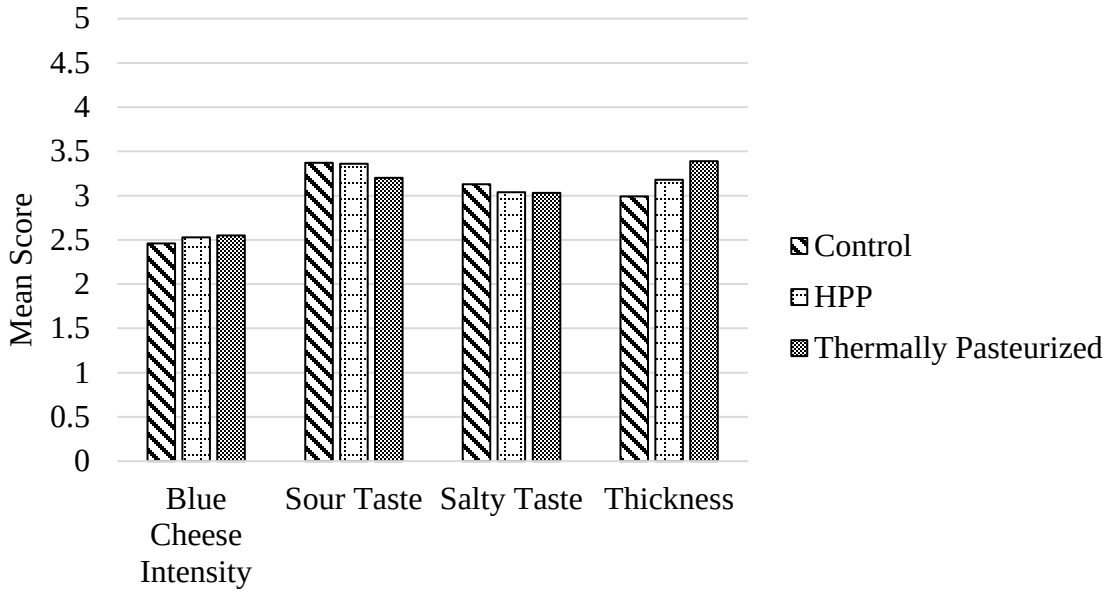


Figure 13. JAR Scores of Various Sensory Attributes of Treated Blue Cheese Dressing (HPP and Thermally Pasteurized) Against Control. A 5-pt. scale (1 – "Not Enough", 3 – "Just-about-right", 5- "Too Much") was used. The HPP variables were treated at 600 MPa for 2 minutes. The control variable was filled at 22 ℃ and its formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. The thermally pasteurized variable was treated at 74 ℃ for 20 minutes and its formulation included 0.1% sodium benzoate and 0.1% potassium sorbate. No statistical differences were demonstrated.

Conclusions. High pressure processing was an effective technology for producing a good quality, relatively high pH at 4.0, blue cheese dressing. As demonstrated in the results, the product exhibited good microbiological, physical, and chemical stability. Clean label was achievable, since yeast and filamentous were inhibited by pressure alone without the use of chemical preservatives. Although *Bacillus velezensis* was found to be a pressure resistant organism that survived the given treatment conditions, it appeared to be unable to grow in the acidic conditions. As is, the blue cheese dressing has the potential for at least a 3-month shelf-life at refrigerated storage or at least 9 weeks at room temperature conditions.

REFERENCES

Appleman, M. D., E. P. Hess, and S. C. Rittenberg. 1949. An investigation of a mayonnaise spoilage. *Food Technol.* 3:201-203.

Claesson, P.M., Blomberg, E., and Poptoshev, E. 2003. Surface forces and emulsion stability. Food emulsions. 4th ed. New York: Marcel Dekker, Inc. p. 257–98.

Difco & BBL Manual. 2008. Manual of microbiological culture media. Sparks, Md.: Becton, Dickinson and Co. Retrieved on May 10, 2018 from http://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/microbiology-solutions-resource-library

Fetzek Jr., A. 1973. The effect of storage temperature and time on the quality of salad dressings. [MSc thesis]. Columbus, Ohio: The Ohio State Univ. 139 p. Available from: The Ohio State Univ. Libraries, Columbus, Ohio, U.S.A.;787096.

Ford L.D., Borwanker, R.P., Pechak D., and Schwimmer, B. 2003. Dressings and sauces. Food emulsions. 4th ed. New York: Marcel Dekker, Inc. p. 525–72.

Han, Y., Xu, X., Jiang, Y., Zhou, G., Sun, X., and Xu, B. 2010. Inactivation of food spoilage bacteria by high pressure processing: Evaluation with conventional media and PCR-DGGR analysis. *Food Research International* 43:1719-1724.

Henney J.E., Taylor C.L., Boon C.S. 2010. Strategies to Reduce Sodium Intake in the United States. Preservation and Physical Property Roles of Sodium in Foods. National Academies Press.

Huck, J.R., Burchett, A., and Huntington, M.K. 2008. Leclercia adecarboxylata in an immunocompetent patient. *J. Med. Microbiol.* 57: 896–898

Mistry B.S., Min D.B. 1993. Shelf life of mayonnaise and salad dressings. Shelf life studies of foods and beverages: chemical, biological, physical and nutritional aspects. Amsterdam, The Netherlands: Elsevier Science Publishing Co. p 409-50.

Muntean, M.V., Marian, O., Barbieru, V., Catunescu, G.M., Ranta, O., Drocas, I., and Terhes, S. 2016. High Pressure Processing in Food Industry - Characteristics and Applications. *Agriculture and Agricultural Science Procedia* 10:377-383.

Nienaber, U., Arora, A., Shellhammer, T.H. 2001. High pressure processed salad dressings: microbiological and rheological aspects. In: IFT Annual Meeting Book of Abstracts; 2001 June 23–27; New Orleans, La. Chicago, Ill.: Institute of Food Technologists. Poster 28-4.

Patterson M.F., Quinn M., Simpson R. and Gilmour A. 1995. Effects of high pressure on vegetative pathogens. High Pressure Processing of Foods. Nottingham University Press, Nottingham. p. 47–63.

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., and Prieto, M. 2011. Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Science and Technology* 44(5), 1251-1260.

Ruiz-Garcia, C., Bejar, V., Martinez-Checa, F., and Llamas, I. 2005. Quesada, E. Bacillus velezensis sp. nov., a surfactant tproducing bacterium isolated from the river Velez in

Malaga, southern Spain. *International Journal of Systematic and Evolutionary Microbiology* 55:191-195.

Smelt, J.P.P.M. 1998. Recent advances in the microbiology of high pressure processing. Trends *Food Sci Technol.* 9: 152–158.

Waite, J.G., Jones, J.M., Turek, E.J., Dunne, C.P., Wright, A.O., Yang, T.C.S., Beckwitt, R., and Yousef, A.E 2009. Production of Shelf-Stable Ranch Dressing Using High-Pressure Processing. *Journal of Food Science*74(2): M83-M93.

Weiss, T.J. 1983. Food oils and their uses. Chichester, U.K.:Ellis Horwood. p. 310.

Zablocki, L.J., Bousman, W.S., Solanki, Y., Milovanovic, S.B., King, A. 2001. Viscosity stable acidic edible liquid compositions and method of making. U.S. patent 6,139,895.

CHAPTER 3

Impact and Future Work

Although the salad dressing market appears to be oversaturated, there is a shortage of clean label products in this category. Most producers are utilizing a combination of sodium benzoate and potassium sorbate as chemical preservatives to prevent yeast and filamentous fungi growth. High-pressure processing is an alternative method for reducing the microbiota of blue cheese dressing to produce a shelf-stable dressing. As previous studies have shown, and the current research demonstrates, high-pressure processing can achieve shelf stability without compromising product quality. The failure modes for these types of products generally include: spoilage, discoloration, rancidity, presence of off-flavors, and emulsion instability. The study was able to show a delay in these failure modes across shelf-life.

While the research demonstrated a 3-month shelf-life of HPP product held at refrigeration and a 9-week shelf-life for product held at room temperature, future studies could extend this testing out even further. As mentioned previously, microbiological spoilage is not the only failure mode, but at the end of 3-month testing, no yeast or filamentous fungi had been detected yet. The only organism present on the media selected, *Bacillus velezensis*, still appeared to be unable to grow. Other modes of failure, including color, particle size, and sensory properties need to be studied past the 3-month mark as well.

The inoculated pathogen studies were only carried through to 10 weeks of shelf-life at the close of the research, however, the FDA's recommended 5-log reduction in *Salmonella, Listeria monocytogenes*, and *E. coli* O157:H7 was still achieved by this point in time. This portion of the study needs to be carried out further. Samples were also only held at 4 °C, so the inoculation study could be performed at room temperature conditions as well to see how the HPP treatment holds.

The HPP conditions were tested at a level commonly used in the industry, 600 MPa for 2 minutes. While future work would not likely lower the length of time pressure is applied for,

there is an opportunity to lower the intensity of the pressure. 600 MPa achieved a 7.5-log reduction in inoculated pathogens, which is conservative of the FDA's recommendation. A lower pressure may not only result in less of an impact on product attributes, but it would result in an energy savings.

Although pH and water activity were briefly investigated in the initial formula optimization study, this is still an area that can be further optimized in future studies. If the water activity could be lowered at all, with HPP still being effective, less gum would need to be used in the formulation. In the sensory studies conducted, 56% of respondents reported that the "sour taste" was just about right. However, 36% reported that the dressing was too sour (Figure 14). If the pH could be raised without impacting the effectiveness of the pressure, this would address some of the dislike for the vinegar taste.

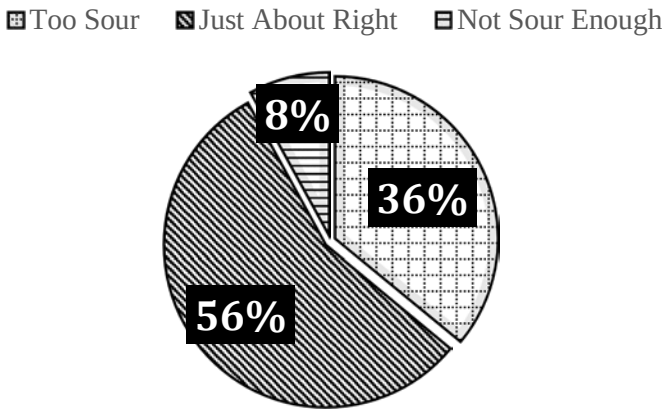


Figure 14. JAR Scale Results for Sour Taste of Blue Cheese Dressing Collected from Sensory Study

A lot of research has reported that when HPP is used as an alternative to thermal pasteurization, it has less of an impact on nutritional properties. Future work could compare the two treatments when applied to blue cheese dressing for breakdown of vitamins and minerals. If thermal pasteurization is indeed more detrimental to these aspects, then another benefit of HPP could be claimed.

The current shelf-life of the studied formulation is comparable to other dressings on the market. Because of this, the product could be taken to commercialization. However, a final step in the research would be to perform a cost analysis and evaluate for feasibility. While sources claim that the use of high pressure adds $0.03-$0.10/unit, this estimate is based off an operation running at full capacity. Future work should evaluate the feasibility of commercializing under HPP to determine if the benefits on the product outweigh any increases in cost per unit.

APPENDIX – RAW DATA

Preliminary Optimization Plating Data

		Before HPP				*After HPP*			
Variable	Rep	TPC (log CFU/g)	APDA (log CFU/g)	MRS (log CFU/g)	Lactic Petrifilm (log CFU/g)	TPC (log CFU/g)	APDA (log CFU/g)	MRS (log CFU/g)	Lactic Petrifilm (log CFU/g)
High vein, pH 4.0, aW 0.95	1	>1.00	5.79	4.09	<1.00	2.57	<3.00	2.48	<1.00
High vein, pH 4.0, aW 0.96	2	>1.00	5.87	4.15	<1.00	2.67	<3.00	2.60	<1.00
High vein, pH 4.3, aW 0.965	1	3.18	5.69	>2.00	<1.00	2.51	<3.00	2.30	<1.00
High vein, pH 4.3, aW 0.965	2	3.16	5.64	>2.00	<1.00	2.56	<3.00	2.30	<1.00
High vein, pH 4.0, aW 0.965	1	2.62	5.75	3.10	<1.00	2.20	<3.00	2.48	<1.00
High vein, pH 4.0, aW 0.966	2	2.63	5.66	3.18	<1.00	2.23	<3.00	2.60	<1.00
High vein, pH 4.3, aW 0.95	1	3.02	5.69	4.09	<1.00	2.26	<3.00	2.48	<1.00
High vein, pH 4.3, aW 0.95	2	3.04	5.56	4.14	<1.00	2.20	<3.00	2.00	<1.00
Low vein, pH 4.0, aW 0.95	1	2.87	5.68	>2.00	<1.00	2.53	<3.00	<2.00	<1.00
Low vein, pH 4.0, aW 0.95	2	2.82	5.7	>2.00	<1.00	2.60	<3.00	<2.00	<1.00
Low vein, pH 4.3, aW 0.965	1	>1.00	5.85	4	<1.00	2.11	<3.00	2.60	<1.00
Low vein, pH 4.3, aW 0.965	2	>1.00	5.92	4.08	<1.00	2.45	<3.00	<2.00	<1.00
Low vein, pH 4.0, aW 0.965	1	3.02	6	3.78	<1.00	2.18	<3.00	<2.00	<1.00
Low vein, pH 4.0, aW 0.965	2	3.99	5.97	4	<1.00	2.46	<3.00	<2.00	<1.00
Low vein, pH 4.3, aW 0.95	1	3.39	6	3.57	<1.00	2.23	<3.00	2.30	<1.00
Low vein, pH 4.3, aW 0.95	2	3.19	5.96	3.65	<1.00	2.20	<3.00	2.00	<1.00

Shelf-Life Plating Data

Week	Treatment	Storage	Rep	TPC (log CFU/g)	APDA (log CFU/g)	MRS (log CFU/g)	Lactic Petrifilm (log CFU/g)
0	Before HPP	N/A	1	3.49	5.48	3.99	<1.00
0	Before HPP	N/A	2	3.61	5.28	4.12	<1.00
0	Before HPP	N/A	3	3.65	5.20	4.88	<1.00
0	HPP	N/A	1	2.34	<1.00	2.48	<1.00
0	HPP	N/A	2	2.61	<1.00	2.60	<1.00
0	HPP	N/A	3	1.85	<1.00	2.48	<1.00
0	Control, No Pres.	N/A	1	3.60	5.23	4.09	<1.00
0	Control, No Pres.	N/A	2	3.51	5.08	3.98	<1.00
0	Control, No Pres.	N/A	3	3.58	5.11	4.08	<1.00
0	Control, Pres.	N/A	1	3.60	5.32	3.95	<1.00

0	Control, Pres.	N/A	2	3.46	5.48	3.98	<1.00
0	Control, Pres.	N/A	3	3.54	5.48	4.16	<1.00
1	Control, No Pres.	22°C	1	4.46	4.85	4.15	<1.00
1	Control, No Pres.	22°C	2	4.51	4.30	4.05	<1.00
1	Control, No Pres.	22°C	3	4.69	4.30	4.30	<1.00
1	Control, Pres.	22°C	1	3.20	3.48	<2.00	<1.00
1	Control, Pres.	22°C	2	3.88	4.00	4.05	<1.00
1	Control, Pres.	22°C	3	4.05	5.66	4.03	<1.00
2	Control, No Pres.	22°C	1	4.47	4.62	4.52	<1.00
2	Control, No Pres.	22°C	2	4.73	4.90	4.92	<1.00
2	Control, No Pres.	22°C	3	4.61	4.59	4.66	<1.00
2	Control, Pres.	22°C	1	4.65	4.49	4.56	<1.00
2	Control, Pres.	22°C	2	4.24	4.28	4.23	<1.00
2	Control, Pres.	22°C	3	4.31	4.28	4.41	<1.00
3	HPP	22°C	1	1.95	<1.00	2.00	<1.00
3	HPP	22°C	2	1.78	<1.00	1.78	<1.00
3	HPP	22°C	3	1.85	<1.00	1.48	<1.00
3	HPP	4°C	1	1.90	<1.00	1.95	<1.00
3	HPP	4°C	2	1.78	<1.00	1.70	<1.00
3	HPP	4°C	3	1.95	<1.00	1.78	<1.00
6	HPP	22°C	1	2.65	<1.00	2.18	<1.00
6	HPP	22°C	2	2.78	<1.00	2.78	<1.00
6	HPP	22°C	3	2.60	<1.00	2.23	<1.00
6	HPP	4°C	1	1.90	<1.00	1.60	<1.00
6	HPP	4°C	2	2.46	<1.00	2.18	<1.00
6	HPP	4°C	3	2.36	<1.00	1.48	<1.00
9	HPP	22°C	1	<2.00	<1.00	<2.00	<1.00
9	HPP	22°C	2	2.00	<1.00	<2.00	<1.00
9	HPP	22°C	3	<2.00	<1.00	2.48	<1.00
9	HPP	4°C	1	<2.00	<1.00	<2.00	<1.00
9	HPP	4°C	2	2.70	<1.00	<2.00	<1.00
9	HPP	4°C	3	<2.00	<1.00	<2.00	<1.00
12	HPP	4°C	1	1.95	<1.00	2.48	<1.00
12	HPP	4°C	2	1.60	<1.00	2.00	<1.00
12	HPP	4°C	3	1.48	<1.00	2.00	<1.00

pH Data

Week	Treatment	Storage	Rep	pH
0	HPP	N/A	1	4.1
0	HPP	N/A	2	4.04
0	HPP	N/A	3	4.1

0	Control, No Pres.	N/A	1	4.03
0	Control, No Pres.	N/A	2	4.09
0	Control, No Pres.	N/A	3	4.12
0	Control, Pres.	N/A	1	4.1
0	Control, Pres.	N/A	2	4.04
0	Control, Pres.	N/A	3	4.02
1	Control, No Pres.	22°C	1	4.13
1	Control, No Pres.	22°C	2	3.96
1	Control, No Pres.	22°C	3	4.09
1	Control, Pres.	22°C	1	4.09
1	Control, Pres.	22°C	2	4.1
1	Control, Pres.	22°C	3	4.2
2	Control, No Pres.	22°C	1	4.07
2	Control, No Pres.	22°C	2	4.08
2	Control, No Pres.	22°C	3	4.09
2	Control, Pres.	22°C	1	4.21
2	Control, Pres.	22°C	2	4.34
2	Control, Pres.	22°C	3	4.21
3	HPP	22°C	1	4.29
3	HPP	22°C	2	4.27
3	HPP	22°C	3	4.18
3	HPP	4°C	1	4.02
3	HPP	4°C	2	4.15
3	HPP	4°C	3	3.92
6	HPP	22°C	1	4.26
6	HPP	22°C	2	4.46
6	HPP	22°C	3	4.35
6	HPP	4°C	1	4.12
6	HPP	4°C	2	4.12
6	HPP	4°C	3	4.2
9	HPP	22°C	1	4.25
9	HPP	22°C	2	4.11
9	HPP	22°C	3	4.36
9	HPP	4°C	1	4.27
9	HPP	4°C	2	4.25
9	HPP	4°C	3	4.17
12	HPP	4°C	1	4.27
12	HPP	4°C	2	4.09
12	HPP	4°C	3	4.26

Water Activity Data

Week	Treatment	Storage	Rep	Aw
0	HPP	N/A	1	0.9556
0	HPP	N/A	2	0.9504
0	HPP	N/A	3	0.948
0	Control, No Pres.	N/A	1	0.9418
0	Control, No Pres.	N/A	2	0.9489
0	Control, No Pres.	N/A	3	0.9531
0	Control, Pres.	N/A	1	0.9442
0	Control, Pres.	N/A	2	0.9503
0	Control, Pres.	N/A	3	0.9457
1	Control, No Pres.	22°C	1	0.9494
1	Control, No Pres.	22°C	2	0.9495
1	Control, No Pres.	22°C	3	0.9508
1	Control, Pres.	22°C	1	0.947
1	Control, Pres.	22°C	2	0.9477
1	Control, Pres.	22°C	3	0.9421
2	Control, No Pres.	22°C	1	0.9377
2	Control, No Pres.	22°C	2	0.9386
2	Control, No Pres.	22°C	3	0.9406
2	Control, Pres.	22°C	1	0.9412
2	Control, Pres.	22°C	2	0.9351
2	Control, Pres.	22°C	3	0.9377
3	HPP	22°C	1	0.9493
3	HPP	22°C	2	0.9488
3	HPP	22°C	3	0.9463
3	HPP	4°C	1	0.9482
3	HPP	4°C	2	0.9496
3	HPP	4°C	3	0.946
6	HPP	22°C	1	0.9301
6	HPP	22°C	2	0.9507
6	HPP	22°C	3	0.9439
6	HPP	4°C	1	0.948
6	HPP	4°C	2	0.9516
6	HPP	4°C	3	0.9444
9	HPP	22°C	1	0.9453
9	HPP	22°C	2	0.9469
9	HPP	22°C	3	0.9445
9	HPP	4°C	1	0.9371
9	HPP	4°C	2	0.9448
9	HPP	4°C	3	0.948
12	HPP	4°C	1	0.9387
12	HPP	4°C	2	0.9401
12	HPP	4°C	3	0.9426

Viscosity Data

Week	Treatment	Storage	Rep	Pa·s
0	HPP	N/A	1	6300
0	HPP	N/A	2	7900
0	HPP	N/A	3	6500
0	Control, No Pres.	N/A	1	6800
0	Control, No Pres.	N/A	2	7700
0	Control, No Pres.	N/A	3	6900
0	Control, Pres.	N/A	1	20000
0	Control, Pres.	N/A	2	7600
0	Control, Pres.	N/A	3	5700
1	Control, No Pres.	22°C	1	6100
1	Control, No Pres.	22°C	2	7300
1	Control, No Pres.	22°C	3	4800
1	Control, Pres.	22°C	1	6000
1	Control, Pres.	22°C	2	6100
1	Control, Pres.	22°C	3	5000
2	Control, No Pres.	22°C	1	5750
2	Control, No Pres.	22°C	2	4900
2	Control, No Pres.	22°C	3	5900
2	Control, Pres.	22°C	1	4350
2	Control, Pres.	22°C	2	3950
2	Control, Pres.	22°C	3	5200
3	HPP	22°C	1	5500
3	HPP	22°C	2	8000
3	HPP	22°C	3	6600
3	HPP	4°C	1	6400
3	HPP	4°C	2	5200
3	HPP	4°C	3	4400
6	HPP	22°C	1	4500
6	HPP	22°C	2	5700
6	HPP	22°C	3	5000
6	HPP	4°C	1	7000
6	HPP	4°C	2	9500
6	HPP	4°C	3	7000
9	HPP	22°C	1	4400
9	HPP	22°C	2	4650
9	HPP	22°C	3	3750
9	HPP	4°C	1	5800
9	HPP	4°C	2	4700

9	HPP	4°C	3	9000
12	HPP	4°C	1	7700
12	HPP	4°C	2	8200
12	HPP	4°C	3	7400

Refractive Index Data

Week	Treatment	Storage	Rep	Refractive Index
0	HPP	N/A	1	1.41891
0	HPP	N/A	2	1.40044
0	HPP	N/A	3	1.39966
0	Control, No Pres.	N/A	1	1.419696
0	Control, No Pres.	N/A	2	1.39426
0	Control, No Pres.	N/A	3	1.40053
0	Control, Pres.	N/A	1	1.39741
0	Control, Pres.	N/A	2	1.39263
0	Control, Pres.	N/A	3	1.40261
1	Control, No Pres.	22°C	1	1.41531
1	Control, No Pres.	22°C	2	1.42256
1	Control, No Pres.	22°C	3	1.39876
1	Control, Pres.	22°C	1	1.39757
1	Control, Pres.	22°C	2	1.39498
1	Control, Pres.	22°C	3	1.3952
2	Control, No Pres.	22°C	1	1.44488
2	Control, No Pres.	22°C	2	1.40573
2	Control, No Pres.	22°C	3	1.40194
2	Control, Pres.	22°C	1	1.40164
2	Control, Pres.	22°C	2	1.39648
2	Control, Pres.	22°C	3	1.39833
3	HPP	22°C	1	1.42099
3	HPP	22°C	2	1.4207
3	HPP	22°C	3	1.40559
3	HPP	4°C	1	1.40639
3	HPP	4°C	2	1.39978
3	HPP	4°C	3	1.39152
6	HPP	22°C	1	1.41934
6	HPP	22°C	2	1.40877
6	HPP	22°C	3	1.406
6	HPP	4°C	1	1.40702
6	HPP	4°C	2	1.40146
6	HPP	4°C	3	1.39313
9	HPP	22°C	1	1.41333

9	HPP	22°C	2	1.40687
9	HPP	22°C	3	1.40158
9	HPP	4°C	1	1.40954
9	HPP	4°C	2	1.39982
9	HPP	4°C	3	1.39915
12	HPP	4°C	1	1.4233
12	HPP	4°C	2	1.42023
12	HPP	4°C	3	1.40635

Particle Size Data

Week	Treatment	Storage	Rep	D [4,3] (μm)	<0.1% (μm)	<0.9% (μm)
0	HPP	N/A	1	335.642	23.635	790.024
0	HPP	N/A	2	259.455	13.808	661.124
0	HPP	N/A	3	310.958	23.872	720.211
0	Control, No Pres.	N/A	1	293.433	22.255	654.157
0	Control, No Pres.	N/A	2	376.668	38.286	814.496
0	Control, No Pres.	N/A	3	345.062	37.995	741.611
0	Control, Pres.	N/A	1	266.129	25.169	610.384
0	Control, Pres.	N/A	2	323.961	31.808	716.127
0	Control, Pres.	N/A	3	285.698	21.582	648.083
1	Control, No Pres.	22°C	1	394.773	49.127	865.468
1	Control, No Pres.	22°C	2	315.291	34.75	719.619
1	Control, No Pres.	22°C	3	305.999	36.857	685.992
1	Control, Pres.	22°C	1	340.338	23.799	780.938
1	Control, Pres.	22°C	2	295.214	22.886	673.517
1	Control, Pres.	22°C	3	330.571	32.487	733.621
2	Control, No Pres.	22°C	1	411.862	32.629	951.932
2	Control, No Pres.	22°C	2	416.704	36.889	948.371
2	Control, No Pres.	22°C	3	499.908	34.307	1130.23
2	Control, Pres.	22°C	1	434.613	46.659	975.073
2	Control, Pres.	22°C	2	334.187	33.845	755.321
2	Control, Pres.	22°C	3	342.159	36.52	765.731
3	HPP	22°C	1	399.59	21.404	918.233
3	HPP	22°C	2	316.296	22.968	740.537
3	HPP	22°C	3	257.679	19.73	606.973
3	HPP	4°C	1	264.302	17.127	638.65
3	HPP	4°C	2	286.322	19.685	676.547
3	HPP	4°C	3	204.072	11.143	673.455
6	HPP	22°C	1	137.758	1.077	341.433
6	HPP	22°C	2	291.389	20.564	677.523
6	HPP	22°C	3	242.112	16.761	575.145

6	HPP	4°C	1	243.03	17.442	578.13
6	HPP	4°C	2	470.946	35.162	1042.829
6	HPP	4°C	3	244.69	11.74	590.32
9	HPP	22°C	1	260.816	13.569	640.184
9	HPP	22°C	2	327.946	21.371	762.695
9	HPP	22°C	3	273.139	16.797	651.664
9	HPP	4°C	1	295.65	21.846	690.035
9	HPP	4°C	2	268.716	16.037	628.76
9	HPP	4°C	3	269.778	15.396	650.213
12	HPP	4°C	1	314.636	22.047	710.509
12	HPP	4°C	2	456.516	31.591	1030.193
12	HPP	4°C	3	380.265	22.494	877.848

NMR Data

Week	Treatment	Storage	Rep	Acetic Acid (%)	Lactic Acid (%)	Citric Acid (%)
0	HPP	N/A	1	0.2008	0.0542	0.0036
0	HPP	N/A	2	0.2203	0.0842	0.0070
0	HPP	N/A	3	0.1206	0.0472	0.0028
0	Control, No Pres.	N/A	1	0.1612	0.0605	0.0025
0	Control, No Pres.	N/A	2	0.1878	0.0557	0.0080
0	Control, No Pres.	N/A	3	0.1764	0.0485	0.0039
0	Control, Pres.	N/A	1	0.1632	0.0464	0.0079
0	Control, Pres.	N/A	2	0.1641	0.0455	0.0019
0	Control, Pres.	N/A	3	0.2073	0.0269	0.0162
1	Control, No Pres.	22°C	1	0.1203	0.0372	0.0028
1	Control, No Pres.	22°C	2	0.1052	0.0520	0.0055
1	Control, No Pres.	22°C	3	0.1322	0.0304	0.0025
1	Control, Pres.	22°C	1	0.1557	0.0409	0.0040
1	Control, Pres.	22°C	2	0.1394	0.0255	0.0035
1	Control, Pres.	22°C	3	0.1570	0.0278	0.0051
2	Control, No Pres.	22°C	1	0.2114	0.0531	0.0024
2	Control, No Pres.	22°C	2	0.1902	0.0537	0.0068
2	Control, No Pres.	22°C	3	0.1848	0.0504	0.0105
2	Control, Pres.	22°C	1	0.1953	0.0656	0.0082
2	Control, Pres.	22°C	2	0.1912	0.0624	0.0105
2	Control, Pres.	22°C	3	0.2403	0.0804	0.0037
3	HPP	22°C	1	0.1003	0.0251	0.0011
3	HPP	22°C	2	0.0952	0.0465	0.0059
3	HPP	22°C	3	0.1138	0.0275	0.0041
3	HPP	4°C	1	0.1207	0.0319	0.0042
3	HPP	4°C	2	0.1122	0.0375	0.0022

3	HPP	4°C	3	0.0857	0.0202	0.0047
6	HPP	22°C	1	0.1516	0.0437	0.0014
6	HPP	22°C	2	0.1094	0.0210	0.0019
6	HPP	22°C	3	0.1464	0.0589	0.0039
6	HPP	4°C	1	0.1114	0.0291	0.0026
6	HPP	4°C	2	0.1632	0.0385	0.0044
6	HPP	4°C	3	0.1439	0.0551	0.0043
9	HPP	22°C	1	0.0605	0.0330	0.0007
9	HPP	22°C	2	0.1043	0.0306	0.0039
9	HPP	22°C	3	0.1248	0.0302	0.0029
9	HPP	4°C	1	0.1305	0.0341	0.0061
9	HPP	4°C	2	0.1082	0.0471	0.0040
9	HPP	4°C	3	0.1057	0.0329	0.0189
12	HPP	4°C	1	0.0798	0.0281	0.0007
12	HPP	4°C	2	0.1213	0.0248	0.0070
12	HPP	4°C	3	0.1089	0.0286	0.0050

Color Data

Week	Treatment	Storage	Rep	L*	a*	b*	ΔL*	Δa*	Δb*	Hue	Chroma
0	HPP	N/A	1	86.41	2.75	12.38	+53.79	-30.35	+10.09	1.35	12.68
0	HPP	N/A	2	86.21	2.22	12.19	+53.59	-30.89	+9.90	1.39	12.39
0	HPP	N/A	3	84.58	1.99	11.05	+51.96	-31.11	+8.76	1.39	11.23
0	Control, No Pres.	N/A	1	84.96	2.36	11.71	+52.33	-30.75	+9.42	1.37	11.95
0	Control, No Pres.	N/A	2	85.9	2.66	11.41	+53.28	-30.61	+9.12	1.34	11.72
0	Control, No Pres.	N/A	3	85.32	2.49	12.51	+52.70	-30.45	+10.22	1.37	12.76
0	Control, Pres.	N/A	1	84.53	2.72	12.22	+51.91	-30.39	+9.93	1.35	12.52
0	Control, Pres.	N/A	2	84.96	2.51	12.14	+52.34	-30.59	+9.85	1.37	12.40
0	Control, Pres.	N/A	3	84.65	2.38	12.30	+52.03	-30.72	+10.01	1.38	12.53
1	Control, No Pres.	22°C	1	80.44	3.22	11.44	+53.30	-23.03	+9.42	1.30	11.88
1	Control, No Pres.	22°C	2	82.57	3.12	10.90	+55.43	-23.13	+9.39	1.29	11.34
1	Control, No Pres.	22°C	3	82.15	2.73	10.90	+55.01	-23.52	+9.39	1.33	11.24
1	Control, Pres.	22°C	1	83.07	2.72	11.09	+55.93	-23.53	+9.57	1.33	11.42
1	Control, Pres.	22°C	2	80.51	2.60	10.78	+53.37	-23.65	+9.26	1.33	11.09
1	Control, Pres.	22°C	3	81.59	3.61	10.86	+54.45	-22.65	+9.34	1.25	11.44
2	Control, No Pres.	22°C	1	82.01	3.65	11.62	+54.87	-22.60	+10.10	1.27	12.18
2	Control, No Pres.	22°C	2	82.01	3.27	11.29	+52.50	-22.98	+9.78	1.29	11.75
2	Control, No Pres.	22°C	3	82.01	3.76	11.09	+55.11	-22.49	+9.58	1.24	11.71
2	Control, Pres.	22°C	1	81.41	3.38	11.08	+53.04	-22.31	+9.69	1.27	11.58
2	Control, Pres.	22°C	2	81.41	3.34	10.37	+54.12	-22.57	+9.79	1.26	10.89
2	Control, Pres.	22°C	3	80.91	3.71	10.54	+53.77	-22.55	+9.02	1.23	11.17
3	HPP	22°C	1	81.71	3.23	10.46	+54.57	-23.02	+8.95	1.27	10.95

3	HPP	22°C	2	81.71	2.98	11.05	+57.06	-23.27	+9.53	1.31	11.44
3	HPP	22°C	3	81.71	3.52	12.38	+55.49	-22.73	+10.86	1.29	12.87
3	HPP	4°C	1	81.8	3.66	12.05	+54.66	-22.59	+10.54	1.28	12.59
3	HPP	4°C	2	84.54	3.38	12.25	+57.40	-22.87	+10.73	1.30	12.71
3	HPP	4°C	3	83.79	3.12	11.56	+56.65	-23.13	+10.05	1.31	11.97
6	HPP	22°C	1	85.53	3.69	12.59	+52.91	-29.41	+10.30	1.29	13.12
6	HPP	22°C	2	85.84	3.34	12.70	+53.21	-29.76	+10.40	1.31	13.13
6	HPP	22°C	3	85.16	3.38	12.56	+52.54	-29.72	+10.27	1.31	13.01
6	HPP	4°C	1	86.43	3.42	13.02	+53.81	-29.69	+10.73	1.31	13.46
6	HPP	4°C	2	84.8	3.48	12.60	+52.18	-29.62	+10.31	1.30	13.07
6	HPP	4°C	3	86.94	3.20	12.56	+54.31	-29.90	+10.27	1.32	12.96
9	HPP	22°C	1	85.65	3.18	12.03	+53.03	-29.93	+9.74	1.31	12.44
9	HPP	22°C	2	84.43	4.07	12.31	+51.81	-29.03	+10.02	1.25	12.97
9	HPP	22°C	3	84.4	3.38	11.99	+51.77	-29.73	+9.70	1.30	12.46
9	HPP	4°C	1	86.14	3.31	12.47	+53.52	-29.80	+10.18	1.31	12.90
9	HPP	4°C	2	87.24	3.46	12.81	+54.62	-29.64	+10.52	1.31	13.27
9	HPP	4°C	3	86.27	3.62	12.73	+53.65	-29.48	+10.44	1.29	13.23
12	HPP	4°C	1	83.58	2.62	11.72	+56.44	-23.63	+10.20	1.35	12.01
12	HPP	4°C	2	80.42	3.03	12.03	+53.28	-23.22	+9.51	1.32	12.41
12	HPP	4°C	3	82.57	2.49	11.46	+55.43	-23.77	+9.94	1.36	11.73

Sodium Data

DM% (##.#)	Na% DM	Cl% DM	Na Salt% DM	Cl Salt% DM	Avg Salt% DM	Na Salt% As	Cl Salt% As	Avg Salt% As
66.9	1.155	1.9	2.96	3.11	3.04	1.98	2.08	2.03
67.6	1.117	1.91	2.86	3.13	3	1.94	2.12	2.03

Inoculated Plating Data

Week	Treatment	Storage	Organism	Log CFU/g
0	Before HPP	N/A	*E.coli*	7.57
0	Before HPP	N/A	*Salmonella*	7.76
0	Before HPP	N/A	*Listeria*	7.52
0	After HPP	N/A	*E.coli*	<1.00
0	After HPP	N/A	*Salmonella*	<1.00
0	After HPP	N/A	*Listeria*	1.85
2	HPP	4°C	*E.coli*	<1.00
2	HPP	4°C	*Salmonella*	<1.00
2	HPP	4°C	*Listeria*	<1.00
6	HPP	4°C	*E.coli*	<1.00

6	HPP	4°C	*Salmonella*	<1.00
6	HPP	4°C	*Listeria*	1.54

Sensory Data

Treatment	Color	Aroma	Visual Texture	Flavor	Texture	Overall
Control	6.95	6.59	6.86	6.52	6.58	6.71
HPP	6.88	6.36	6.76	6.44	6.64	6.66
Thermally Pasteurized	3.97	5.73	3.47	6	4.16	4.88